The Effects of Environmental Exposure on Human Scalp Hair Root Morphology

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1. Abstract

The relevance of hair roots as evidence has been long established by forensic scientists, especially in areas of trace evidence examination and molecular biology. It has been shown that microscopic examination of hairs at the proximal or root end can reveal information about its growth phase, determine if hair may have been unnaturally shed, or show evidence of decompositional changes at the root end. Other types of analyses with hair roots include sex-typing and nuclear DNA extraction to yield short tandem repeat profiles, which aid in identifying the subject possessing the hair. In order to glean this information from hair roots, it is important that the root be intact and unaltered by the external environment. Unfortunately, a dearth of work on hair root degradation exists. Some of the work that has been done includes examination of postmortem hair samples to assess the frequency of root banding patterns, as well as experimentation with conditions that give rise to a decomposed root. This project serves to explore the results of exposure to different indoor environments on antemortem hair root morphology.

In this study, a set of five experiments examined the effects of environmental exposure on the morphology of human scalp hair roots from anagen and telogen growth phases. Anagen phase represents active hair growth and is characterized by amorphous morphology and incomplete keratinization of the root end. Telogen phase represents the quiescent phase of hair growth, distinguished by complete keratinization of the root end. Human scalp hairs, submitted by volunteers, were examined and classified as being of the anagen or telogen growth phase. They were subsequently exposed to indoor environments of air exposure (negative control), soil burial, and pond water immersion. Examination of these hairs was performed with brightfield light microscopy. Because of the incomplete keratinization of anagen roots, it was postulated that they would be more susceptible to changes from environmental exposure. The results of all five

experiments showed more advanced morphological alteration in anagen versus telogen roots in the soil and water environments. The first experiment revealed certain patterns of change that arose after environmental exposure in four different subjects. The patterns consisted of banding, darkening, shriveling, and fraying. There was also evidence of adhering debris, including the presence of microorganisms, to the roots. The second experiments examined the progressive changes in hair root morphology over time in soil and water exposure. Changes in anagen roots initially began as apparent shriveling and advanced to erosion of root structure, banding, or complete obliteration of the root structure. This progression began as early as 24 hours of exposure and was advanced at 4 days of exposure. Telogen roots were minimally affected by exposure to yield slight fraying and darkening of the root bulb even with the longest exposure times. In later experiments, sterilization of hairs and environments was introduced to determine whether nonsterile conditions produced earlier and more advanced degradation in hairs as compared to sterile conditions. This suggested that hair root morphology changes are partly caused by microbial sources from the environment.

In conclusion, this study demonstrates the vulnerability of anagen roots to degradation after soil and water exposure. Certain patterns of change arose in these roots that suggested a breakdown in the structural integrity of the hair at the proximal end. While the causes for these degradation patterns are unknown, preliminary work here has demonstrated that microbial action is a contributor. The banding patterns that have arisen in anagen hair roots bear a striking resemblance to published images of postmortem root banding. Since this issue has arisen in legal cases, there is a need for further research to determine the causes of this banding pattern in both antemortem and postmortem hair samples.

2. Introduction

Hair, though seemingly innocuous and generic, contains a great deal of information, and thus, is valuable as evidence in forensic science. A strand of hair can aid in identification, be an indicator of substance abuse, associate a person with a certain place, and can display decompositional changes at the root end. Research has uncovered many of these uses for hair and continues to discover more as hair evidence is becoming more crucial in determining the outcome of many legal proceedings.

One issue of concern that has been minimally explored is that of human scalp hair root degradation. Unfortunately, this little-known topic has been under scrutiny in past criminal cases. Upon researching the topic of human scalp hair degradation, it was found that recent studies have been conducted on hair shaft breakdown upon exposure to different environments (Chillé 1990, Kundrat et. al 1989, Rowe 1997). These studies showed that hair shafts, deemed as being resilient and not easily destroyed due to the presence of keratin, are actually vulnerable to attack and alteration by certain decomposers such as fungi, bacteria, and insects. After exposure to these biological agents, microscopic changes in hair shaft morphology appeared in distinct patterns such as internal tunneling and exterior erosion. Other studies on hair morphology have included examination of the anagen roots of postmortem specimens to microscopically analyze the characteristic appearance of postmortem root banding (Linch and Prahlow 2001). This was further investigated when postmortem hair samples were exposed to varying environmental factors to monitor their influence on appearance of banding patterns (Wager Collins 1996).

While hair *shaft* degradation has been demonstrated in the literature, it is of a different concern as that of hair *root* degradation. Hair shafts are completely keratinized, or filled with keratin protein. Thus, the factors responsible for degradation involve the ability to breakdown

keratin, whether physically, chemically, or biologically. Hair roots, though, have varying degrees of keratinization, depending on their stage in the growth cycle. Because keratin is a biochemically stable and extremely durable protein (Voet and Voet 1995), which affords protection and strength, it is believed that regions of incomplete keratinization are more vulnerable to changes in morphology upon environmental exposure. This is especially apparent in the phenomenon of postmortem root banding, a pattern of degradation of the internal hair root structure. The banding pattern is observed in hair samples that have undergone decomposition in the follicle. It is present in the anagen root, usually at a region distal to the root end. This is the region where the hair is embedded in the scalp and close to the hair follicle; it consists of cellular and tissue matter as an immature hair strand.

Very little data exists on the topic of hair root degradation resulting from environmental exposure. The aim of this study is to evaluate the effects of environmental exposure on antemortem human scalp hair root morphology. Anagen hairs, which are hairs that are actively growing, possess a gradient zone of keratinization at the proximal tip, with keratinization levels increasing as one travels distally to the permanent hair shaft. Their counterparts, telogen hairs, or hairs at the end of the growth stage, possess a uniform level of keratinization from root to tip. Looking at the incompletely keratinized anagen hairs as a site for morphological changes, experiments are featured where both anagen and telogen hairs were subjected to various environments and microscopically examined thereafter. Photomicrographs exhibiting the changes are featured; controlled experiments were conducted to find the cause of the degradation. This knowledge has important implications for the observation and understanding of changes in hair root morphology, especially in comparing those changes that are produced as a result of intra-follicular postmortem decomposition with those rendered by extra-follicular environmental exposure. This is a controversy that has appeared in past legal proceedings. The results of this

study will benefit the forensic science community, especially in the areas of examination and interpretation of hair evidence.

3. Review of the Literature

3.1. Hair Root Anatomy

Hair is an appendage of the integumentary system, located in various places of the body, such as the scalp, axillary, pubic, facial, nasal, and digital regions. The present discussion is for hairs located on the scalp, although hair anatomy is extremely similar in different body regions. Hair is a strong, fibrous material containing interesting arrangements of cells that are comprised largely of keratin protein. Interspersed among these cells are pigment granules of melanin, and air or fluid-filled vacuoles called cortical fusi (Bisbing 2002). Each hair shaft can be further divided into three layers, which are revealed by microscopic analysis.

Exteriorly located is the cuticle layer, made of overlapping, colorless, flattened cells, averaging about six layers thick. This protective layer, appearing as shingles on a roof, covers the entire hair shaft and is adjacent to the cortical layer. The cortex is the main layer, occupying 88% of the hair shaft (Kaszynski 1985). This portion is a mélange of elongated, fused, and hardened cells, melanin granules, and cortical fusi. The arrangement of these components varies from person to person, and is used in microscopic comparisons in forensic casework (Robertson 1999). The innermost layer is the medulla. This exists as a central column of cells running through the cortex, along the central axis of the shaft (Bisbing 2002, Harding and Rogers 1999). Under transmitted light, the medulla sometimes appears as a dark region, when it is filled with air (Bisbing 2002). The medulla's appearance is also highly variable. It is classified as either absent, continuous, discontinuous, or fragmented by the Scientific Working Group for Materials Analysis (SWGMAT 2000). This characteristic manifests as a dark central canal that is either missing, complete from nearly tip-to-tip, randomly present, or dashed, respectively. Also microscopically

visible are the distal tip, and the proximal, or root, end. The remainder of the discussion will be limited to literature concerning the root end.

Hair emanates from an organ in the skin called the hair follicle. In the human scalp, this organ is anywhere from 1-4 mm below the stratum corneum, or outermost, layer of the epidermis (Robbins 1985, Harkey 1992). Hair growth is divided into different zones based on the regions of cell synthesis, cell differentiation, and protein synthesis that take place in the hair follicle. These zones are given various names by different authors (Robbins 1985, Harkey 1992). The germinative region of the hair follicle, or site of cell synthesis, is localized in a region called the follicle bulb. In this bulb is a deeply indented region that contains vascularized connective tissue called the dermal papilla (Forslind 1990). This region supports mitotic activity of the nearby follicle matrix cells, which are part the germinative compartment of the follicle. The germinative compartment, while responsible for hair growth, also dictates the pigmentation of hair, as melanocytes are present in this region. Eumelanin (black-brown pigment) and pheomelanin (yellow-red pigment) are produced by the melanocytes and give color to the differentiating cells of the hair shaft (Henrikson et al. 1997).

Between the bulb and middle of the hair follicle is the differentiating region. Cells in the upper bulb differentiate into layers of follicle and shaft, which produce a column of concentrically arranged cell layers. The outer root sheath is the outermost epithelial cell layer, continuous with the basal layer of the epidermis. Moving inward is the inner root sheath. This layer contains cells which keratinize, or fill with keratin protein, near the level of the sebaceous gland. The inner root sheath consists of three layers. Adjacent to the hair shaft is the cuticle, moving outward are the Huxley and Henle layers, which are next to the outer root sheath. The innermost layer is the hair shaft. This layer consists of three types of cells, cuticle, cortical, and medullary cells. Cuticle cells are squamous cells, which form the cuticle, or outermost layer of the hair shaft and intermingle with

the cuticular cells of the inner root sheath. Cortical cells are cuboidal cells, which give rise to the cortex of the hair, and contain alpha-keratin. The medullary cells are present as a central canal and consist of vacuolated or air-filled cells (Henrikson 1997).

Also present near the hair root are two other anatomical structures. Smooth muscle bundles located near the hair follicle and dermis of the skin are called arrector pili muscles which, upon contraction, give the appearance of goose flesh. Sebaceous glands are present near the interface of the hair follicle and beginning of shaft, which is just below of the epidermal layer of the scalp (Robbins 1985). These glands provide oily secretions for the hair and skin (Grollman 1974). Collectively, the hair follicle and sebaceous gland are known as the pilosebaceous unit (Harkey 1992).

3.2. Hair Root Physiology and Hair Growth

As scalp hair extrudes, the hair follicle undergoes a cyclic growth cycle, alternating between active growth and quiescence (Kaszynski 1985). Unlike animals, the human hair growth cycle is asynchronous. Each hair follicle participates in its own cycle, independent of those adjacent to it (Chase 1965). This cycle consists of three growth phases, called anagen, catagen, and telogen. During the first stage of growth, or the anagen phase, hair is actively growing from the follicle (Bisbing 2002). At this stage, the follicle is at maximum length, sometimes four to five millimeters long (Harding and Rogers 1999). The growth rate for the hair shaft being produced at the follicle is 0.3 to 0.4 mm/day. These actively growing, or anagen, hairs comprise 80-90% of the hair (Harding and Rogers 1999). The next growth phase, one of transition between activity and quiescence, is called catagen. At the follicular level, this phase is characterized by cessation of cell division, atrophy of the follicle, and keratinization at the base of the hair shaft (Harkey 1992). Only 2% of hairs or less are in the catagen phase. The last stage of hair development, the telogen

phase, is characterized by inactivity. The follicle dramatically reduces to about half of its original length. The hair shaft remains attached to the remnants of the follicle and is called a club hair due to its characteristic club-like appearance at the proximal end. At this stage, the hair is completely keratinized from the root bulb onward. Because of the shrunken hair follicle, telogen hairs are easily shed during mechanical removal, such as grooming, or by a newly emerging hair. About 10-18% of hairs are in the telogen phase (Bisbing 2002). After the telogen phase ends, the hair follicle returns to active growth in the anagen phase.

3.2.1 Anagen Physiology and Structure

The anagen phase constitutes the largest part of the growing cycle. In the human scalp, typical anagen phases can last anywhere from two to four years (Chase 1954). Harding and Rogers (1999) describe the subdivisions of anagen into different stages, deemed proanagen, mesanagen, and metaanagen, where metaanagen is the phase of hair protrusion from the scalp. Proanagen is further divided into four substages. During these four substages: I:) the cells of hair follicle are actively dividing, II:) the follicle grows around the dermal papilla and cells enter their first stages of differentiation into the inner root sheath, where keratinization occurs, III:) follicle grows to three to six times its resting length, the inner root sheath has formed, and pigmentation begins, and IV:) mitosis occurs in the follicle bulb, and synthesis of the hair cortex and medulla has begun. At mesanagen, the hair follicle has the appearance of an onion bulb, which encloses the dermal papilla, and firmly attaches to the epidermis. Lastly, at metaanagen, hairs are seen above the skin surface and continue to grow without any further structural changes to the follicle, pigmentation also slows down.

In the anagen growth phase, the hair is deeply embedded in and firmly mounted to the scalp. Normally, hairs from the anagen phase are only shed unnaturally, usually by plucking,

pulling, or other types of force (Petraco et al. 1988, Robertson 1999). Depending on the speed of removal, sheaths of tissue may or may not be attached (Petraco et al. 1988). An anagen hair root has a very amorphous, flattened, ribbon-like appearance (Robertson 1999). The shape of the proximal end has various morphologies such as long, narrow, and slightly pigmented, or widened at the base containing a highly pigmented (seen in images from Petraco et al. 1988, Harding and Rogers 1999). Images of anagen hairs collected from this study exhibited a high degree of morphological diversity. Some examples are featured in Figure 1.

3.2.2 Catagen Physiology and Structure

At the termination of active hair growth, a brief period of transition, known as the catagen phase, begins (Harkey 1999). This phase is extremely short, in relation to the anagen and telogen phases, lasting approximately two weeks. During the catagen phase, structural and morphological changes occur within the follicle, converting the active organ to one of quiescence. Pigmentation of the hair ceases as the melanocytes change structure and halt the genesis of melanin (Parakkal 1990). Another noteworthy feature is that the once extended follicle regresses to less than one-third of its original length as cell division stops and the cell contents are digested by a number of enzymes, namely acid phosphatases and esterases, to give rise to a strand of epithelial cells (Kaszynski 1985). This strand connects the dermal papilla to the hair germ (Harding and Rogers 1999). In the upper part of the follicular bulb, cells continue to differentiate into components of the permanent hair shaft, namely the cortical cells (Parakkal 1990). This nodal, "brush-like" region becomes what is called the club hair (Harkey 1992, Harding and Rogers 1999). As this area continues to grow, keratinous filaments and rootlets form, stemming from the cortical cells, which serve to mount the hair to the surrounding epithelial tissue of the follicle (Kaszynski 1985).

Due to the presence of the aforementioned rootlets, hairs in the catagen phase remain attached to the remains of the follicle (Harding and Rogers1999). Upon being pulled from the scalp, the structure of the root appears "often non-pigmented and brushlike, sometimes surrounded by an epithelial sac" (Harding and Rogers 1999). Petraco et al. (1988) describe the root morphology as having "a dried-up root sheath... and a club-shaped base". Because hairs in this phase are in transition, it appears that the root morphology is highly variable. Hairs in this phase sometimes more closely resemble those from the late stages of active growth while others appear more like those in early stages of latency. Linch (1998) states that whole mount light microscopic distinction between late anagen and early catagen, and late catagen and early telogen, is not always clear.

3.2.3 Telogen Physiology and Structure

After the physiological changes of the transitional catagen phase have terminated, the stagnant phase of hair growth, called telogen begins. Scalp hair follicles spend about 10 weeks or 2.5 months in this resting phase (Harkey 1992). It is important to note that at this phase, the hair is at its most stable, mature state (Harding and Rogers 1999). The club is separated from the ball of cells in the dermal papilla by an epithelial layer. The cells of the germinative compartment are absent and those cells in the lower part of the follicle are mitotically inactive (Montagna and Parakkal 1974). A telogen root is superficially embedded in the scalp, just below the level of the sebaceous gland. It is only mounted to the scalp at the club root (Bisbing 2002), since tissue surrounding the hair has atrophied and retracted (Harding and Rogers 1999).

Late stage telogen hairs are easily dislodged by a minimal amount of force. Most do not fall out by themselves, but are shed through grooming processes such as combing and washing of the hair (Harding and Rogers 1999). Their appearance at the root end is that of a "cotton swab"

(Linch et al. 1998) with a rigid, oval-shaped tip that tapers as one extends along the axis of hair. The root tip is hardened and bulbous with little or no adherent follicular tissue (Bisbing 2002). Other features of the telogen scalp hair at the root end are the following: no pigment granules above the root bulb, no medulla on the lower portion of the hair shaft near the root, and a higher concentration of cortical fusi just above the root bulb (Petraco et al.1988). Due to the residual presence of keratinized rootlets from the catagen phase, sometimes, upon pulling, a telogen hair can possess a remnant of these rootlets, which is identified by hair examiners as the club nipple, or the germinal nipple (Linch et al. 1998). Figure 2 shows some images of the telogen hair root from hairs collected during the study.

3.3. Keratin and Levels of Keratinization within a Growing Follicle

Voet and Voet (1995) surveyed the biochemical details of keratin. Keratin is a mechanically durable and chemically stable protein found in all higher vertebrates. Appendages such as skin, nails, hair, horns, scales, feathers and quills are made principally of keratin (Baden 1990). In mammals, this fibrous protein is classified as α-keratin, due to its coiled protein structure. Electron microscopy and x-ray diffraction have served to elucidate the structure of keratin, especially in structures such as human hair. Examinations have revealed that hair, which is mainly made of α-keratin, consists of a hierarchy of protein structures. In the dead cells making up the hair strand, tightly packed cylindrical structures, called macrofibrils (2000 Å in diameter) and microfibrils (80 Å wide), are found. These long fibrils are oriented parallel to the long axis of the hair.

At the molecular level, it is revealed that keratin exists as a helical protein polymer. The rudimentary substructure of keratin is a dimeric polypeptide (~450 Å long) consisting of a Type I

and a Type II keratin--called the intermediate filament (Harding 1999) chain twisted in parallel into a left-handed coiled coil. The next substructure of keratin, called a protofilament, consists of two staggered and antiparallel rows of associated head-to-tail dimers. When two protofilaments dimerize, a protofibril is formed. A microfibril results from the coalescence of four protofibrils. The largest structural feature of keratin fibers is the macrofibril. This is composed of tightly packed microfibrils; the number of which is variable. The complex and intimate construction of keratin protein combined with chemical cross-linking, such as rigid disulfide bonds, results in a strong, and insoluble material.

Keratinization is the process in which a growing hair shaft fills with keratin protein superstructure (Baden 1990), thereby rendering strength and durability to a hair. Within a follicle, this process occurs at successive structurally identifiable levels (Kaszynski 1985). It is important that these zones be identified for later discussion about the degrees of keratinization of hairs in different growth stages. The seven zones mentioned below describe the area of the follicle at which different stages of keratinization occur (Kaszynski 1985).

1. Matrix: in lowest part of follicle bulb
2. Pre-elongation zone: at level of greatest diameter of bulb
3. Cellular elongation zone: near neck of bulb
4. Pre-keratinization zone: just beyond neck of bulb
5. Keratogenous zone: at a slightly higher level of the follicle
6. Pre-cortex
7. Mature cortex: in shaft of mature hair

For simplification purposes, these seven zones are usually grouped into four zones: 1) cell proliferation and differentiation zone at the base of the bulb, 2) keratin gene expression zone in the upper bulb, 3) keratogenous zone where hardening of the hair fiber occurs, and 4) the zone of

inner root sheath degradation, also the site of the mature hair (Harding and Rogers 1999). These zones in the hair suggest that there is a significant portion of the developing hair shaft, between the bulb and its neck, that is unkeratinized in the early growth stages of the hair.

Kaszynski (1985) and Roberts (1999) described the stages of keratin development in a growing hair. During the late anagen phase in hair growth, certain ultrastructural features arise in the matrix cells at the base of the follicle bulb, which are associated with keratin synthesis. These features consist of ribosomes and highly developed endoplasmic reticula, suggestive of protein synthesis. In the cytoplasm of these cells, there is an absence of filamentous protein material, due to the immature state of synthesis. However, in the cellular level above the matrix, the cytoplasm contains both free and cell membrane-bound keratin filaments in early stages of extension.

Traveling into the higher levels of the follicle, near the neck of the bulb, cells undergo differentiation into layers of the outer root sheath, inner root sheath and permanent hair. In Henle's and Huxley's layers of the inner root sheath, granules of trichohyalin (a form of keratohyalin, which is a precursor to keratin) appear and cells, their nuclei and cytoplasmic components, also elongate parallel to the long axis of the follicle. More-developed keratin filaments parallel to the long axis of the follicle are also seen in this region. These filaments contain sulfhydryl (S-H) groups. Acid phosphatase activity, which may be responsible for breakdown of cell nuclei and organelle material, is also found in this pre-keratinization zone. As the cells move through the neck of the bulb, the keratin filaments increase in size and widen to form macrofibrils. In the keratogenous zone, the protein fibrils mature into alpha-keratin. This is evident with polarized light microscopy, as alpha keratin is brilliantly anisotropic (Kaszynski 1985). Images of this are featured in Figure 21.

In the pre-cortical portion of the hair, in addition to the sulfhydryl groups in the keratin filaments, monobasic and dicarboxylic amino acids may also exist. It should be noted that pre-keratin swells in distilled water and dissociates in urea (Kaszynski 1985), suggesting that is not as

durable as mature keratin, and is susceptible to morphological changes. As the pre-cortex matures into the cortex, salt bridges from between the aforementioned amino acids, oxidation of S-H groups generate disulfide (S-S) bonds, which produces coalescence and hardening of the keratin fibrils. Only in the zone of mature hair are keratin filaments completely cross-linked, providing strength and durability to the hair (Bisbing 2002). Linking these fibers of keratin, among other components in the hair, is an intercellular matrix called cell membrane complex, a cellular "cement" of sorts (Harding 1999). It should be understood that keratin and melanin pigment are not the only components in hair. The relative chemical composition of hair can be described as 90% protein (including 17 of 20 amino acids), 15-35% water, 1-9% lipids, and 0.25 to 0.95% minerals (Harkey 1993). Moreover, past the site of the follicle, the hair is considered to be inert, and biologically inactive, or "dead", contrary to what is portrayed by many of the cosmetic and hair care product corporations (Harding and Rogers 1999).

3.4 Hair Degradation

In searching for literature about hair root degradation and related topics, a mixed amount of information was revealed. A great deal of work has been done surveying changes in the hair shaft, upon exposure to environments such as soil, water, and indoors resulting from things such as fungal (Kundrat and Rowe 1989; DeGaetano et al. 1992; Kupferschmid et al. 1994), and insect attack (Chillé 1990). Changes in hair shafts have also been observed as a result of chemical modification (Choudhry et al. 1983). Brief mention was given to the appearance of a burned hair shaft and the morphological changes that result (Petraco and De Forest 1993). Only a limited amount of work has been done in the area of the hair root morphology. Linch (1998) described the morphological changes in hair roots resulting from enzymatic exposure and claimed that the morphological changes were the same in microscopic appearance as hairs from a decomposing

human scalp. The results of another study (Linch and Prahlow 2001) indicated that hair roots do not decompose after fresh removal from the scalp and exposure to the outside elements. The two studies were only conducted in relation to a phenomenon known as postmortem root banding (discussed in section 3.5). The sources listed above were helpful in guiding the experimental design and demonstrated that further research is needed in areas of hair root degradation (Petraco et al. 1988). Below, the studies that were found are elaborated upon, demonstrating the valuable information that was gained about hair degradation and morphological changes, as well as the forensic applications to these discoveries.

Deterioration of physical evidence, specifically trace evidence such as hair, is an important phenomenon that forensic scientists must face when examining evidential and exemplar specimens. If, for example, an exemplar hair specimen is collected from a person's scalp only to be compared to an evidential specimen that has been subjected to morphological changes due to immersion in water, this has many implications on the quality of the comparison. For a hair examiner to conclude that two hairs could have originated from the same source, the exemplar and evidential hairs must not only show the same characteristics but also the same degree of variation in those characteristics. Work done by Kundrat and Rowe (1989) and Chillé (1990) demonstrated how environmental exposure could change the morphology of hair shafts. In the Kundrat study, hair samples were subjected to agricultural soil burial for various time intervals and microscopically exhibited various stages of fungal attack: adhering hyphae, tunneling, and perpendicular fragmentation. This was caused by keratinophilic fungi that secrete keratinase, an enzyme that digests keratin. The Chillé study placed hairs in various locations indoors for an eight-month time period. Digestion of the shaft by both fungi and insects was microscopically evident; it manifested as bite marks, tunneling, shredding.

Other studies correlated certain morphological changes with attack by a particular agent, be it microbial or chemical. Recognizing specific traits in hair that appear as a result of biodeterioration or chemical adulteration could either decrease or increase the probative value of the hair. Kundrat and Rowe (1989) and DeGaetano et al. (1992) demonstrated that tunneling of a hair shaft is something that can be classified as being evidence of fungal attack. Moreover, in different specimens the pattern of digestion differs. Some tunneling appears as very narrow, transverse fissures, perpendicular to the hair shaft; others branch and run parallel to the long axis of the shaft, and others have a conical appearance (Rowe 1997). Choudry et al. (1983) demonstrated the changes in hair shaft surface morphology as a result of chemical exposure. Different hair samples were exposed to proteolytic, denaturing, or disulfide bond-reducing agents. Mercaptoacetic acid at pH 3.85 produced dramatic surface results. This disulfide bond-reducing agent produced a characteristic restructuring of the hair shaft surface, as indicated by SEM analysis. Treatment with mercaptoacetic acid also served as a way to discriminate between hairs of different individuals because of the high degree of intra-individual consistency in the chemically induced topological changes in the hair surface.

When classifying hairs as being of animal or human origin, environmental exposure could efface the hairs to such a high degree that making this classification could be impossible. Kupferschmid and Rowe et al. (1994) demonstrated that, upon immersion of hairs in water and burial in soil, cuticular scale patterns were in some cases, completely eradicated, as revealed by SEM. This work shows the decreased value of hair evidence that has structural morphological changes as a result of environmental exposure. Since cuticular scale patterns in animals and humans differ, this is an important step in classification of unknown hairs. When scale patterns are so completely obliterated, or highly altered, a hair examiner may have difficulty speciating the hairs.

The results of these studies indicate that, although hair is highly resilient and not easily destroyed by environmental conditions (Kupferschmid et al. 1994), certain structural and morphological changes can arise as a result of microbial and/or chemical exposure. The degradation studies conducted on hair shafts revealed that hair cuticular patterns could be effaced by microbial agents in soil and water, thus complicating determination of species. They also pointed out certain telltale signs of fungal and insect attack and chemical exposure due to changes in internal structure and surface topology, such as tunneling into the shaft, and cavitation and blebbing of hair surface. Lastly, these morphological and structural changes in environmentally degraded samples make it imperative that known and questioned samples originate from similar conditions in order to facilitate the evaluation of a link between the two. Unfortunately, there is a paucity of work on hair root morphology changes as a result of environmental exposure. As mentioned earlier, only two limited studies have been performed on hair roots. Since degradation studies on hair shaft have provided valuable information to hair examiners, a vast amount of data could be gathered from studies conducted specifically on hair roots. There is no doubt that this data could also yield important information.

3.5 Postmortem Root Changes

The existence of postmortem root banding, "dead man's root end", or "putrid roots" has been well documented in the literature by many forensic hair examiners (Bisbing 2002, Tafaro 2000, Linch et al. 1998, 2001, Petraco et al. 1988, and Deadman 1990), as well as the Committee on Forensic Hair Comparisons (SWGMAT 2000). This phenomenon is understood as manifesting in one of two ways. The first sign of a putrid, or decomposing, root is a black band or postmortem band about 0.5 mm from the tip of the hair's root, and about 2 mm below the skin surface (Petraco et al. 1988). This band actually consists of an area of tightly arrayed, parallel longitudinal spaces

filled with air that appears black when viewed under transmitted light, and bright under incident illumination (Tafaro 2000, Petraco et al. 1988). The other change associated with a decomposing root usually occurs after the hair has decayed further or has been pulled from the hair follicle. Due to a separation through the root, the hair proximal end usually appears as a brushy root due to its frayed, brush-like appearance (Tafaro 2000).

While the exact cause for the appearance of the decomposing root is unknown, a theory has been developed. It is understood that if one dies and begins to decompose, the hairs embedded in the skin will undergo postmortem changes. Although the earliest appearance of incipient postmortem root banding has appeared in 8 hours since death, the pattern is usually indicative of longer exposure (Petraco et al. 1988). Banding has been observed in anagen root configurations, and in the zone of banding, it appears that the hair is more susceptible to degenerative changes such as autolysis and/or microbial action that appear in decomposition. For microbial mechanisms, it is postulated that there is a competition between the degree of keratinization of the hair and the access that microbes have available. This could be explained by the degree of keratinization and disulfide bonding. Above the banding zone, hairs are more fully keratinized and strengthened by disulfide linkages. Beneath the banding zone, the hair is afforded protection by being embedded in the skin (Petraco et al. 1988). Reports of banding configurations in telogen hairs have not been reported the literature. However, it has been stated that the keratin of the telogen club can be likened to that of human nail and is thus resistant to decompositional changes in the surrounding tissues (Linch and Prahlow 2001).

Studies have explored the evidential value of postmortem hair roots. The frequency of appearance in deceased persons from various causes of death, postmortem intervals, ages, races, sexes, and conditions which may encourage the formation of postmortem bands have been investigated (Linch et al. 1998, Linch and Prahlow 2001, Tafaro 2000, and Wager Collins 1996). In

two homicide cases, decompositional changes in the root allowed associations to be made between evidential and examplar hairs (Tafaro 2000). Other work revealed that time since death, or postmortem interval, cannot be determined from the appearance of postmortem bands or brushy roots due to the irregularity of their appearance (Linch and Prahlow 2001). Postmortem bands can only indicate to an examiner that the hair was shed by a deceased person (Bisbing 2002). In a study by Wager Collins (1996), scalp plugs containing hairs from recently deceased persons were subjected to soil and sand at different depths and different temperatures. This study revealed that elliptical, darkened band patterns developed in all scalp-embedded specimens except for those stored at freezing temperatures. Banding appeared earliest (24 hours from exposure) at room temperature and was maximal at 3 days, as compared to a 7-day exposure for the onset of banding in refrigerated samples. This suggests that presence inside the follicle and increased temperatures favor the formation of decompositional changes.

Postmortem root decompositional changes are mentioned in this literature review because they are features that have become issues in legal proceedings. Because of the cryptic nature of the mechanism behind postmortem root banding, the lack of research on this topic (Petraco et al. 1988), and the varied morphological appearance and location of the bands (Wager Collins 1996, Linch et al. 1998), hair examiners can only speculate about this phenomenon, why it occurs, etc. One case arose in which two forensic scientists were testifying on the presence of banding patterns near the root structure of some evidential hairs. Due to the location of the bands, more proximal to the tip, there was a dispute as to whether the root changes were caused by postmortem decomposition, or environmental exposure of free hairs. As mentioned earlier, a small study was conducted by Linch and Prahlow (2001) to test the results of environmental exposure on hair roots. Hairs of telogen and anagen phases from a living person were subjected to outdoor air exposure in winter and summer months, for 42 and 14 days, respectively. The researchers

examined these hairs under transmitted light microscopy to reveal that no "decompositional" changes existed. This study was used in the paper to make a generalization that "hair roots do not decompose after fresh removal from the scalp and exposure to the outside elements". However, this was an inadequate study; more experiments should have been performed in order to draw such a conclusion.

The present study represents a more comprehensive evaluation of environmental exposure on hair root morphology. It is comprised of five different experiments. The details of each experiment are given below including title, objective, and hypothesis. Refer to Figure 23 for a flow chart featuring a summary of the methods employed in this study.

Experiment #1: Indoor exposure of anagen and telogen hair roots to air, water, and soil environments.

Objective: To assess the impact of environmental exposure on anagen and telogen hair roots.

Hypothesis: Hair roots will be susceptible to changes after environmental exposure. Morphological changes will be more advanced in anagen roots, due to their incomplete keratinization.

Experiment #2: Indoor exposure of anagen and telogen roots of one subject to air, water, and soil environments and the impact of exposure on each hair over time

Objective: To reinforce the hypothesis that anagen hair roots are more susceptible to morphological changes upon environmental exposure. To measure morphological changes over time. To study the frequency of certain morphological changes.

Hypothesis: Hair root morphological changes will be produced after only a short time of exposure to soil and water. With increased exposure time, these changes will progress.

Experiment #3: Indoor exposure of anagen and telogen roots of one subject to air, soil, and water environments with sterilization control

Objective: To determine if the changes in anagen and telogen hair roots are microbial. To assess the origin of the microbes as belonging to the hair root, or to the environment.

Hypothesis: Changes will be evident in the anagen hair roots that have not been autoclaved. Changes may be present in autoclaved hairs placed in experimental environments, but will arise more slowly than those in the un-autoclaved conditions.

Experiment #4: Indoor exposure of anagen and telogen roots of one subject to soil and water, both sterile and nonsterile

Objective: To compare hair root morphology of hairs kept in sterile environments versus those kept in nonsterile environments. To ascertain whether results are different in each environment.

Hypothesis: Hairs sterilized in sealed environments will exhibit no change in hair root morphology.

Experiment #5: Minimal disturbance of hairs in environments and its effect on morphological change in hair roots

Objective: To assess whether mechanical disturbance creates any background changes in hair root morphology that could be confused with being attributed to environmental exposure.

Hypothesis: Hairs that are not handled as frequently, via removal and placement back into environments with each microscopic examination, will not exhibit as severe changes in root morphology as those that are disturbed frequently.

4. Materials and Methods

In order to conduct this study, it was necessary to collect scalp hair samples from human subjects. The John Jay College of Criminal Justice Institutional Review Board was consulted and approval to conduct this study was granted.

4.1 Experiment #1: Indoor Exposure of Anagen and Telogen Hair Roots to Air, Water, and Soil Environments

Description of Subjects/Collection of Hair Specimens:

Four volunteers were used in this study. They were assigned a number from 1-4. Subject 1 was a 25-year-old Caucasian male with dark brown hair, roughly 2-3 inches in length. Subject 2 was a 24-year-old Caucasian female with brown and white hair, roughly 12-13 inches in length. Subject 3 was a 23-year-old Asian female with dark brown hair, nearly 15 inches in length. Subject 4 was a 22-year-old Caucasian female with dark blond hair, approximately 6 inches in length. None of the subjects' hairs were chemically processed. Subjects were instructed to comb and pull hair and place combed and pulled hair in separate sealable plastic baggies for classification. 52 combed hairs and 52 pulled hairs were collected from each subject.

Classification of Hair Roots:

An Olympus SZH stereomicroscope, at varying magnifications, was used to view the hair root structures more easily to familiarize oneself with the conformations of the anagen and telogen phases. Telogen roots appeared as white-colored, rigid bulbous masses, similar to the shape of a

cotton swab. Anagen roots were highly variable in structure, sometimes possessing a tissue sheath, with an amorphous structure. After initial familiarity was gained, the gross distinctions in roots were made with the naked eye.

Immobilization of Hairs:

Thirteen hairs of anagen or telogen origin were placed onto clear packaging tape (2" wide) with proximal (root) ends protruding ~1.5" from top of tape edge, detailed labels were placed in the middle of the tape mount, and tape was folded onto itself, securing the hairs. This step was completed for each subject, type of hair root, and environment. This was done to immobilize the hairs on an easily retrievable medium. In total, 32 tape mounts were created. Each of the four subjects had two mounts for each type of hair root, multiplied by 4 environments.

Description of Environments/Hair Placement:

Four environments were designed for this study to simulate a sealed setting (negative control), air exposure, water immersion, and soil burial. The airtight setting was merely an opaque sealed plastic container. The air-exposed, or ambient, environment was a plastic lid. Water was collected from a local park from a small, freshwater pond and stored in a 64-ounce container with lid kept ajar to encourage air exchange. Algal growth was noted at time of collection. The soil was also collected from a local park, placed in a 4-liter flowerpot, and kept moist. Fungal growth in the soil was noted at time of collection. Saran wrap was placed over the pot to keep the soil moist. The aforementioned tape mounted hair samples were placed into each environment. These mini-environments were stored at room temperature, ~72° F, on a large windowsill that had daily exposure to light.

Hair Collection from Environments:

Hairs were exposed to selected environments for a two-month time period and were collected at 8 different time points. At each collection point, hairs were removed from environments and proximal ends were cut from tape mounts. Permanent slide mounts of the cut hairs were prepared with Cargille™ Meltmount (n_D=1.539). This refractive index is close to that of the hair cuticle, thus reducing the diffraction of light at hair edges, so that internal structures can be discerned more easily (Linch et al. 1998, Petraco et al. 1988). A collection of reference standard slides was prepared which contained anagen and telogen hairs from each subject that were never environmentally exposed.

Microscopic Examination and Analysis of Hair Roots:

A bright field, compound light microscope (Leica BF 200) was used to microscopically examine the hair roots from each subject, environment, etc. Magnifications of 100x and 200x were used to look at traits such as root shape, root size, root color, shaft color, tissue sheaths, and presence of abnormalities. Negative control hairs were first examined to assess the traits common to each subject. Next, environmentally exposed hairs were examined and any deviations from typical hair root morphology were noted. Sketches of hair roots were made, and later, digital photomicrographs of slides were taken.

4.2 Experiment #2: Indoor Exposure of Anagen and Telogen Roots of One Subject to Air, Water, and Soil Environments and the Impact of Exposure on Each Hair over Time

Description of Subject/Collection of Hair Specimens:

Subject 4 from Experiment #1 was used in this experiment. The subject's hair had been chemically treated; however, root ends possessed new hair growth that was un-treated. Hairs were collected by pulling and combing as described in experiment #1. Three hairs of each type were used for each of the three environments.

Classification of Hair Roots:

Roots were classified as being anagen or telogen with macroscopic examination.

Immobilization of Hairs:

Anagen and telogen root-bearing hairs were affixed to separate pieces of tape for each environment. Tape mount method is as described in experiment #1. In total, six mounts tape mounts were made—three for anagen hairs, three for telogen hairs.

Microscopic Analysis of Hair Roots:

Initial examination of each hair was conducted with a transmitted light microscope (Olympus BH-2) at 100x magnification to assess root appearance and characteristics before environmental exposure. Photomicrographs of hair roots were taken with a Nikon CoolPix 4500 digital camera to document root appearance.

Description of Environments/Hair Placement:

Three of the four experimental environments described in experiment #1 were used to simulate water, soil, and air-exposed environments. An airtight environment was not used. Soil and water were collected from a local park and stored in 32 oz clear, plastic containers with lids kept ajar. Ambient environment was as described in experiment #1. Tape mounts bearing anagen and telogen hairs were placed into each environment. The environments were also stored indoors in a windowless office with standard fluorescent light at room temperature, ~72° F.

Microscopic Examination of Hairs after Environmental Exposure:

Hairs were exposed to environments for a seven-month time period. Hairs were examined at five different time points during the first month and then examined once more at 7 months from time zero. At each time point, hairs were removed from environments. Soil-buried hair samples were lightly rinsed with distilled water to remove any adhering particulate matter. Hairs immersed in water were patted dry with paper towel. They were then temporarily mounted in R.A. Lamb Histoclear™ (n_D=1.471, determined with Bausch & Lomb Abbe Refractometer) onto slides and examined under transmitted, brightfield light microscopy. Histoclear consists of limonene, a distillate of orange oil, which is normally used as a clearing agent for histological slides, but can also be used as an alternative to xylene as a temporary mounting medium, although the refractive index is substantially lower (xylene n_D=1.522). Each mounted hair was photomicrographed with a Nikon CoolPix 4500 digital camera at 100x, and sometimes 200x magnification. After microscopy, hairs were removed from mounts and carefully placed back into the experimental environments.

Analysis of Hair Images:

At the completion of the experiment, hair images were placed side by side and compared to assess the types of changes, if any, that arose in the roots. Intra-hair and inter-hair comparisons were made to answer the following questions. Did more change take place with anagen roots versus telogen roots? At what time point did the earliest change occur? What was the predominant change in the hair roots? Which environment produced the greatest amount of change?

Repetition of Experiment:

This experiment was repeated with a few slight changes. A six-month exposure time was used. Six of seven examinations were done over a three-month time period, and then at six months from time zero, the seventh microscopic examination was conducted. This experiment was conducted again to see if the changes in hair roots were reproducible.

4.3 Experiment #3: Indoor Exposure of Anagen and Telogen Roots of One Subject to Air, Soil, and Water Environments with Sterilization Control

Sterilization of Hairs and Experimental Environments.

This experiment was designed as described in experiment #2 except sterilization of hairs and environments was introduced. Two sets of soil and water environments, as well as three sets of anagen and telogen-phase hair roots were delegated for sterilization. Before hairs were placed into experimental environments, those environments and hairs set aside for sterilization were placed in a pressure cooker for thirty minutes at 10 pounds per square inch (psi). Hairs were sterilized after being immobilized onto tape and put into a small autoclave-safe plastic container.

Two sets of soil and water environments, as well as three sets of anagen and telogen hairs remained unsterilized. The experiment is outlined in the following table. Table 1 features four different types of hair roots: sterile and nonsterile anagen and telogen roots. These hair roots were placed in 5 different types of environments: ambient indoor air exposure, and sterile and nonsterile soil and water burial and immersion, respectively. Each type of hair root was subjected to each type of environment.

Table 1: Design of Sterilization Experiment:

	Hair Roots			
Environments	Autoclaved Anagen	Autoclaved Telogen	Un-Autoclaved Anagen	Un-Autoclaved Telogen
	ambient	ambient	ambient	ambient
	autoclaved soil	autoclaved soil	autoclaved soil	autoclaved soil
	un-autoclaved soil	un-autoclaved soil	un-autoclaved soil	un-autoclaved soil
	autoclaved water	autoclaved water	autoclaved water	autoclaved water
	un-autoclaved water	un-autoclaved water	un-autoclaved water	un-autoclaved water

Microscopic Examination/Analysis of Hair Roots:

Hairs were exposed to their experimental environments for approximately one month. Over this period of time, hairs were examined at seven different times. As described in the previous experiment, hairs were temporarily mounted, microscopically examined, and photomicrographed. Analysis of the hair roots was conducted as mentioned in experiment #2. Comparisons of hair root morphology were made in hairs that were sterilized and those kept nonsterile. Environmental sterility was also factored into the analysis. See discussion about maintenance of environmental sterility.

Notes about Autoclaving Hairs:

To ascertain whether the extreme temperatures encountered during autoclaving would create background changes in hair roots, a mini-experiment was conducted with thirteen hairs of combined anagen and telogen origin. These hairs were microscopically examined in temporary Histoclear™ mounts before autoclaving. They were then subjected to the following sterilization conditions, either 30 min at 10 psi with a pressure cooker or 15 min at 20 psi (solid cycle) with an autoclave. Temperature reached about 121° Celsius in the autoclave. Hairs were housed in a plastic container during the sterilization. Hairs were microscopically examined after the sterilization. It was found that these sterilization methods did not change the appearance of the hair root morphology.

4.4 Experiment #4: Indoor Exposure of Anagen and Telogen Roots of One Subject to Soil and Water, both Sterile and Nonsterile

Method:

Hairs and environments were prepared as previously mentioned in prior experiments. Moreover, hairs were microscopically examined and photomicrographed pre-environmental placement to document their first appearance. Hairs (both anagen and telogen roots) and environments were divided into two sets, one for autoclaving, and the other to remain un-autoclaved. The former hairs were placed into soil (appeared semi-moist) and water environments that were housed in sealed glass jars. They were then pressure cooked at 30 min at 15 psi. Hairs were stored in the soil and water (there was no ambient environment) environments for three weeks (21 days). They were then microscopically examined once to avoid introducing contamination.

4.5 Experiment #5: Minimal Disturbance of Hairs in Environments and its Effect on Morphological Change in Hair Roots

Method:

Hairs and experimental environments were prepared as described previously. However, once hairs were placed in experimental environments, they were not examined repeatedly, only once to minimize the amount of mechanical disturbance by the examiner. Hairs were exposed for a total of 8 days. Hair roots were microscopically examined before and after environmental exposure with temporary mounts and were documented with digital photomicrography, as previously described.

Refer for Figure 23 for a summary flow chart of the methodology used in each experiment.

5. Results

Experiment #1:

Assessment of Patterns of Morphological Alteration in Anagen and Telogen Roots After Environmental Exposure

Images of slides from each subject, and environment, were examined to assess the types of patterns that emerged that were not present in the reference standards. Reference standard hair roots of anagen and telogen phase exhibited a wide degree of variation in their root morphology. However, each hair exhibited root morphology characteristic of its type. Anagen hairs possessed amorphous roots sometimes enveloped by a tissue sheath. The color of the roots ranged from very dark to colorless. Tips of roots appeared tapered and thin or widened at the tip with a pigmented root base. Telogen roots exhibited more uniformity in their structure. Each tip was shaped with the aforementioned "cotton swab" morphology, with a bulbous root wider than the diameter of the hair shaft. Colors and sizes of each root varied from subject to subject. See Figures 1 and 2 for examples. Detailed results of hair root exposure to different environments follow.

Airtight Environment

Since the negative control environment was shielded from air exposure, it was expected that the morphology of the hair roots would be unaffected. This was indeed the case. All of the anagen and telogen hairs collected and microscopically examined from this environment over the two month period exhibited a normal appearance (data not shown), as described above.

Ambient Environment

All hairs exposed to this indoor air experimental environment for the two-month period did not exhibit any deviations from the normal root morphology of the anagen and telogen root phases (data not shown).

Soil Environment

Hairs that were buried in the moistened soil environment exhibited a variety of different patterns at the proximal end that were not characteristic of the reference standard and negative control hair roots. In anagen hair roots, the most common traits were the following: black banding at various distances (0.1-0.35 mm) from the root tip, darkening at the extreme tip, brush like tips, pointed tips, and missing root structures. Less common traits encountered were shriveling or wrinkling of the root, and adherence of particulate matter from the soil. In telogen hairs, the most common traits were darkening of the root bulb and adherence of particulate matter. A less common trait was a pointed root end and a brushy root end. These patterns sometimes appeared in combination with other patterns, for example, a pointed root end appeared with a band distal to it. See images in Figure 4 illustrating the traits mentioned above. The appearance of these traits varied from subject to subject. Table 2 follows which describes the most common traits in each subject ordered from the most to least frequently occurring, organized by root type.

Table 2: Root Morphology Patterns Observed After Soil Exposure

Soil Environment

Subject	Anagen Root Patterns			Subject	Telogen Root Patterns	
#1:	banding	darkened end	pointed tip	#1:	particulate	darkened bulb
#2:	banding	brushlike end		#2:	darkened bulb	brushlike end
#3:	darkened tip	banding	brushlike tip	#3:	darkened bulb	particulate
#4:	banding	missing root	pointed tip	#4:	darkened bulb	particulate
	most frequent →		least frequent		most frequent →	least frequent

Water environment:

Hairs stored in the water environment also had patterns at the root end that did not resemble those in the standard reference or negative control hairs. In anagen hairs, the most common patterns that appeared were the following: darkening, banding, and adherence of debris, including microorganisms. Less common traits were pointy ends, and shriveling. In the telogen hairs, the most common traits that arose were darkening of the root bulb, and adherence of debris, including microorganisms. Less common traits were normal root appearance (unchanged morphology as compared to reference standards), brush like root tip. See examples of anagen and telogen hair roots after water immersion in Figures 5 and 6. The appearance of these traits varied from subject to subject. Table 3, which follows, describes the most common traits in each subject ordered from the most to least frequently occurring, organized by root type.

Table 3: Root Morphology Patterns Observed After Water Exposure

Water Environment

Subject	Anagen root patterns			Subject	Telogen root patterns	
#1:	darkened end	debris, microorganisms adhering	misshapen morphology	#1:	debris, microorganisms adhering	darkened bulb
#2:	darkened end	black banding	pointed end	#2:	darkened bulb	debris, microorganisms adhering
#3:	darkened end	debris, microorganisms adhering	-	#3:	darkened bulb	debris, microorganisms adhering
#4:	black band	shriveling	darkened end	#4:	darkened bulb	debris, microorganisms adhering
	most frequent →		least frequent		most frequent →	least frequent

Association of Morphology Changes with Time

Hairs were exposed to the environment for a total of 2 months. In anagen hair roots, patterns of darkening, banding, and erosion of root structure became more enhanced with time. The earliest appearance of a change in typical anagen root morphology was after 1 day of exposure to the water environment. The latest appearance of a change in root morphology was after 4 days of soil or water exposure. By two months of exposure, all anagen hairs collected from the soil environment exhibited dark bands near the roots. The anagen hairs collected from water displayed darkening near the root tip at two months of exposure. Darkening of telogen root bulbs became more enhanced with time, but not in all subjects. At two months time, most telogen hairs from soil exhibited a darkened bulb, yet some maintained normal root morphology. Telogen hair collected from water at two months exposure possessed more altered root structure than those from soil. Each subject's root bulb had narrowed in diameter; possessed adherence of microorganisms, and some possessed advanced darkening.

Anagen vs. Telogen Root Morphology After Environmental Exposure

While both types of hair roots were affected by environmental exposure, it appeared that anagen root morphology was affected to a greater degree as compared to telogen roots. Telogen root morphology, although slightly changed, was still discernible in all samples after two months of environmental exposure. Anagen root morphology, on the other hand, was altered to a large extent. In many cases, environmental exposure had completely removed the root end.

Experiment #2:

Assessment of Successive Root Morphology Changes Over Time Caused by Environmental Exposure

In the duplicate experiments that were performed, definite changes in root morphology were displayed in the test samples, that is, those stored in soil and water, with the most prominent changes occurring in anagen roots. Additionally, these changes became more advanced over time. In the ambient environment, hair root characteristics in both anagen and telogen samples were preserved for the entire six and seven month periods. A detailed description of results from both soil and water environments follows.

Soil Environment

Because of the large volume of data generated from the duplicated experiments, a summary of morphological changes is presented. Figures of certain hairs are featured, as well. In anagen hairs, soil exposure drastically altered the root structure. After four days of exposure, some of the anagen root ends were missing and reduced to a point. Subsequent changes to the root were minor after this, perhaps because all of the un-keratinized portions of the root had been digested by certain components in the soil. A common progression of changes in the anagen root structure were as follows: shriveling, erosion of the root structure to yield a pointy end, then further

erosion, vastly reducing the length of the proximal root end and producing a rounded-off end. The hair in Figure 7 is an example of the slow progression to complete degradation of the root structure, ~1mm of the hair root was obliterated. Another pattern of morphological change observed was shriveling, and then slow darkening to produce a cluster of elongated black streaks at varying distances from the root tip, resembling a slight band. Unfortunately, two of the hairs exposed to the soil environment were irretrievable since the proximal ends were missing from the tape mount.

In contrast to anagen roots, telogen roots were not as altered by soil exposure. As in experiment #1, extreme ends of telogen root bulbs exhibited a slow progression of darkening and a bit of erosion of the root bulb edges, thus narrowing the root's diameter, and/or producing brushy edges. Over time, two telogen samples also succumbed to the soil activity and were irretrievable from tape mounts for further examination.

The obvious changes in anagen root morphology began around four days of exposure. Slight changes in the hair root, such as apparent shriveling, occurred earlier than this. However, the changes that occurred at four days produced notable differences. In telogen hairs, root alteration did not begin to manifest until around eight or nine days of exposure. See images in Figure 8 of a telogen root exposed to soil over time.

Water Environment

The hairs immersed in the water environment exhibited a variety of changes in morphology at the proximal (root) end. In anagen roots, water exposure drastically altered morphology. In contrast to soil exposure, anagen roots continued to change with increased time spent in water. The progression of changes was usually as follows: initial darkening, shriveling, and erosion of the rounded tip to yield a pointed tip. Next, elongated black streaks appeared very proximal to the root end. Figure 20 shows a magnified example of these black streaks in a hair root. The length of the

hair root appeared drastically reduced; the remnants of the tip flattened out, and developed a darkened hue due to the high concentration of elongated streaks in the hair. Figure 9 exhibits this progression.

Telogen roots exposed to water exhibited minimal alterations in morphology. Some samples possessed some darkening of the tip of the bulb, disappearance of germinal nipples (the tag of tissue attached to the apex of the root bulb-see Figure 2 for example), or slight fraying of the bulb edges. The progression of changes, if they existed, was as follows: disappearance of germinal nipple first, then fraying of the bulb edges, and darkening of the bulb edges. Unlike the anagen roots, telogen root bulbs were still discernible after water exposure due to the minimal changes in the root morphology. See Figure 10 for an example of the progression of changes within a telogen root hair after water exposure.

The earliest change in the anagen hair root was apparent after one day of water exposure; this was usually shriveling. The more drastic changes, such as obliteration of the proximal root tip were present after 3 or 4 days of water exposure. Changes in telogen roots started appearing after eight days of water immersion and continued on for the entire duration of exposure. However, the rate of change seemed very slow. In contrast to soil-exposed hairs, all water-exposed hair samples were retrievable through the entire duration of the experiment.

See Table 4 follows, which features results of experiment #2. Results from both experiments, or batches, of experiment #2 are shown. This table summarizes the conditions of experiment #2 such as the time points, days since time zero, hair types, and environments. Most importantly, however, are the traits that appeared at the root end of these hairs as a result of environmental exposure. Codes are featured for each trait, which are described in a key beneath the table. A gradient of gray color is featured in the tables, which correlated with progression of

changes. Where changes in the hair root became more enhanced, the gray color in the table is darker.

Table 4: Summary of Results in Experiment #2: Root Morphology Changes over Time as a Result of Environmental Exposure

Experiment #2 Batch 1

	Examination days	14-Mar	18-Mar	23-Mar	31-Mar	13-Apr	25-Oct
	Time point	1	2	3	4	5	6
	Days Since Time Zero	0	4	9	17	30	221
Hair Type	*Environment*						
anagen	ambient	nc	nc	nc	nc	nc	nc
	soil	nc	shriv	shriv	reduced brushy	erosion	missing
	water	nc	point	point, dark	dark	erosion	streaky
telogen	ambient	nc	nc	nc	nc	nc	nc
	soil	nc	nc	nc	dark		
	water	nc	nc	dark	dark	darker	darker

Experiment #2 Batch 2

	Examination Days	24-Apr	25-Apr	27-Apr	2-May	16-May	28-Jul	25-Oct
	Time point	1	2	3	4	5	6	7
	Days Since Time Zero	0	1	3	8	22	94	181
Hair Type	*Environment*							
anagen	ambient	nc	nc	nc	nc	nc	nc	nc
	soil	nc	shriv	shriv	point	erosion	reduced	missing
	water	nc	shriv	erosion	point, dark	erosion	dark, reduced	point, reduced
telogen	ambient	nc	nc	nc	nc	nc	nc	nc
	soil	nc	no g.n.	no g.n.	debris	dark	darker	missing
	water	nc	nc	nc	no g.n.	f. edge	f. edge	f. edge

*Gray scale in table cell darkens where changes at root end were more enhanced

Description of codes

Anagen
band= area of darkening at or near root end
brushy= root end was frayed, brush-like
dark= darkened root end
erosion= eroded root end
missing= hair missing from tape mount
nc=no change from time zero
point= root end appeared pointed
reduced= reduced length as compared to time zero
shriv= apparent shriveling at root end
streaky= elongated black streaks visible near root end

Telogen
dark= darkened bulb tip
debris= adhering debris
f. edge= frayed bulb edges
missing=hair missing from tape mount
nc= no change from time zero
no g.n.=germinal nipple missing

Experiment #3:

Comparison of Sterile and Nonsterile Hairs in Sterile and Nonsterile Environments

This experiment yielded results that were similar to the other previous experiments. In sum, anagen roots developed more advanced morphological changes as compared to telogen roots. Patterns that arose in anagen hair roots consisted of shriveling, darkening, banding and fraying. In telogen roots, morphological changes consisted of darkening and fraying of root bulb edges. However, introduction of sterilization to hairs and environments hampered the root morphological changes, and seemed to lessen their severity. A detailed comparison between autoclaved and un-autoclaved hairs in each environment is described below.

Ambient Environment

No morphological changes were observed in either the anagen or telogen autoclaved and un-autoclaved hairs through the one-month duration of ambient exposure. See Figure 11, which feature anagen and telogen roots that were exposed to ambient conditions.

Autoclaved Soil Environment

Both autoclaved and un-autoclaved anagen hairs that were buried in autoclaved soil exhibited root changes. However, the latter hairs began showing root changes after four days of exposure as compared to the former hairs, which showed signs of change after eight days of exposure. After 33 days of exposure, all hairs were still present on the tape mounts. Both types of hairs exhibited advanced degrees of degradation by this time. See images that compare the progression of changes in anagen root morphology of autoclaved and un-autoclaved hairs in Figure 12.

Autoclaved and un-autoclaved telogen hair roots were also altered as result of burial in autoclaved soil. However, as above, the autoclaved hairs exhibited changes after a longer incubation time, eight days, as compared to two days for the un-autoclaved hairs. In short,

autoclaved telogen hairs did not exhibit as much change as compared to those that were not autoclaved. At 33 days of exposure, all hairs were still present on the tape mounts. The un-autoclaved telogen hair roots displayed more advanced degree of degradation as compared to the autoclaved telogen hair.

Autoclaved Water Environment

Autoclaved and un-autoclaved anagen hairs immersed in autoclaved water exhibited changes in their root morphology. Autoclaved anagen hairs showed very minimal changes, which did not begin to manifest themselves until between eight and eighteen days of exposure. Un-autoclaved anagen hairs displayed a more advanced degree of morphological change which became evident at one to two days of exposure. Figure 13 displays autoclaved and un-autoclaved anagen roots that were stored in autoclaved water.

Autoclaved and un-autoclaved telogen roots immersed in water were also affected morphologically. Autoclaved hairs began to show changes at eight days of exposure; while un-autoclaved hairs displayed changes earlier, at two to four days of exposure. The latter hairs also possess a more advanced degree of degradation than the former hairs.

Un-Autoclaved Soil Environment

Autoclaved and un-autoclaved anagen hairs buried in un-autoclaved soil displayed root morphology changes. In the sterile hairs, changes manifested at four days of exposure as shriveling and then advancing to severe darkening of the root tip by 33 days. The nonsterile hairs produced root changes after one or two days of exposure. Root changes began with shriveling and progressed slowly. Unfortunately, at eighteen days, the un-autoclaved hairs were irretrievable from tape mounts so the final changes in the root were unobservable.

Telogen hairs, both autoclaved and un-autoclaved, possessed changes in the root structure after burial in un-autoclaved soil. However, as mentioned above, at the eighteen-day

exposure time point, the un-autoclaved hairs were missing from the tape mount and were thus irretrievable. In the autoclaved hairs, roots exhibited changes starting after eighteen days of exposure. These changes manifested as fraying of the root bulb edges and progressed to slight darkening in the bulb. In the un-autoclaved hairs, changes began after one to two days of exposure. They began as removal of the germinal nipple from the root bulb and progressed to darkened, frayed bulb edges by eight days of exposure. Any changes after these were unobserved due to results of being irretrievable.

Un-Autoclaved Water Environment

Anagen hair roots that were both autoclaved and un-autoclaved were affected by immersion in un-autoclaved water. The sterilized hairs displayed root morphology changes at eight days of exposure while those that remained un-sterilized exhibited changes earlier, at two days of exposure. These changes consisted of initial shriveling and shrinking of the root tip, followed by darkening and or development of elongated black streaks near the root tip clustered in the center of the hair.

Autoclaved and un-autoclaved telogen hair roots were changed after exposure to this environment, as well. Autoclaved hair roots possessed changes such as fraying of bulb edges between four to eight days of immersion, while the un-autoclaved hairs displayed these changes earlier at one to two days.

Table 5 displays a summary of results from this large experiment. This table features hair root types, environments, and their status as having been autoclaved or not. All examination time points are shown. The most important feature of this table is the description of traits, their initial appearance, and their progression. The traits are given in code, as in Table 4, but the code description is the same for both tables.

Table 5: Experiment #3: Progression of Root Morphology Changes in Autoclaved and Un-Autoclaved Conditions

				Date:	31-Jul	1-Aug	2-Aug	4-Aug	8-Aug	18-Aug	2-Sep
				# Days since time 0	0	1	3	4.5	8	18	33
				Time point	0	1	2	3	4	5	6
Hair Type	Autoclaved?*	Environment	Autoclaved?*		Traits observed microscopically						
Anagen	yes	ambient	No		nc	nc	nc	nc	nc	nc	nc
		soil	YES		nc	nc	nc	nc	shriv	dark	band
			No		nc	nc	nc	dark	shriv	shriv, band	dark, band, point
		water	YES		nc	nc	nc	nc	nc	nc	shriv
			No		nc	nc	shriv	erosion	streaky	streaky band	point, dark
	No	ambient	No		nc	nc	nc	nc	nc	nc	nc
		soil	YES		nc	shriv	shriv	streaky	streaky shriv	band, shriv	band, shriv
			No		nc	shriv	shriv	shriv	band	root gone	missing
		water	YES		nc	dark, streaky	shriv, streaky	band, reduced	same	shriv	reduced
			No		nc	shriv	shriv, dark	same	dark	streaky	dark, reduced
Telogen	YES	ambient	No		nc	nc	nc	nc	nc	nc	nc
		soil	YES		nc	nc	nc	nc	nc	no g.n., dark	same
			No		nc	nc	nc	f.edge	same	f. edges, dark	dark
		water	YES		nc	nc	nc	nc	nc		dark
			No		nc	nc	nc	brushy edge	erosion	erosion, dark	same
	No	ambient	No		nc	nc	nc	nc	nc	nc	nc
		soil	YES		nc	nc	nc	nc	dark	dark	dark
			No		nc	nc	nc	g.n. shriv	no g.n.	missing	missing
		water	YES		nc	nc	dark	f.edge	f. edge	f. edge	dark
			No		nc	nc	f. edge	f.edge	f. edge, dark	dark	same

Experiment #4:

Comparison of Anagen and Telogen Hairs Housed in Sealed, Sterile Environments with Those Housed in Open, Nonsterile Environments

Soil Environment

Anagen and telogen hairs stored in the sealed, sterile soil environment were mainly free of changes at the root end. The only exception to this was in one telogen hair, in which the germinal nipple appeared shorter after incubation. Those hairs stored in the nonsterile, open soil environment, however, exhibited many changes. These changes manifested at the root end, as well as in the shaft. Erosion of the root end, as well as extensive tunneling of the shaft were observed. Macroscopic hair digestion was observed as some hairs reduced to extremely short proximal ends that had to be removed from the tape mounts to be microscopically examined. Figure 14 compares anagen hairs stored in sterile and nonsterile soil. Figure 15 compares telogen hairs stored in sterile and nonsterile soil.

Water Environment

Anagen hairs stored in both the sterile, sealed environments, as well as the nonsterile, open environments exhibited changes at the root end. In anagen hairs, this appeared as shriveling, and darkening. It was equally advanced in both types of water environments. Telogen hairs, however, appeared more changed after exposure to the un-autoclaved water. These roots possessed darkening, and fraying at the bulb edge. Figures 16 and 17 feature anagen, and telogen, hair roots, respectively, that were stored in sterile and nonsterile water.

A summary of results follows in Table 6. This shows a comparison between hair root morphology changes that have occurred as a result of sterile and nonsterile experimental environmental exposure. This table gives a description of hair root appearance before and after

exposure, as well as a rating of changes that have occurred. A scale for these ratings is given beneath the table.

Table 6: Experiment #4: Assessment of Root Morphology Change after Sterile and Nonsterile Experimental Environmental Exposure

Hair Type	Environment	Sterile?*	Before Exposure	After Exposure (21 days later)	Morphological Change Rating**
Anagen	soil	S	light colored root end, tendrils	same appearance	0
		NS	pointed end, tendrils	eroded end, tendrils missing	2
	water	S	pointed end	root end curled, shriveled	2
		NS	light colored, widened root end	darkened, pointy root end	3
Telogen	soil	S	bulbous root with germinal nipple	germinal nipple shortened	1
		NS	bulbous root with germinal nipple	root end missing, tunneling in shaft	4
	water	S	bulbous root with germinal nipple	germinal nipple missing	2
		NS	bulbous root	darkened, frayed edges	3

*S=yes, NS=no

**0=no change, 1=slight change, 2=moderate change, 3=advanced change, 4=obliteration of root

Experiment #5:

Repeated Placement and Removal of Hairs through Examination Does Not Create Artificial Changes in Root Morphology

Hairs exposed to the ambient environment exhibited no changes in root morphology. Patterns of change witnessed in anagen and telogen hairs stored in both soil and water environments were advanced, as in earlier experiments that required repeated microscopic examination. Hairs were exposed for eight days and were left undisturbed during exposure. Figure 18 shows anagen hair before and after environmental exposure; Figure 19 shows telogen hair before and after environmental exposure.

Refer to Table 7 below for a summary of results from this brief experiment. This table shows that changes in morphology were observed after 8 days of exposure. Hairs were only examined one time, thus reducing mechanical processing endured by the hairs through repeated examination.

Table 7: Experiment #5: 8 Days of Experimental Environmental Exposure with Minimal Disturbance

Hair Type	Environment	Morphological Change Present?
Anagen	ambient	no
	soil	yes
	water	yes
Telogen	ambient	no
	soil	yes
	water	yes

6. Discussion

The five sets of experiments that were conducted in this study revealed a great deal about the changes that hair roots experience during environmental exposure. Concurrently, a great deal of method development and optimization took place. This study slowly evolved to yield more of an elaborate survey about the vulnerability of hair at the root end. Below is a brief discussion of the results of each experiment, and the problems that occurred during the study.

Experiment #1:

Patterns of Morphological Alteration in Anagen and Telogen Roots After Environmental Exposure

This initial experiment was conducted to determine if hair roots exposed to different environments would exhibit changes in root morphology as a result of some factor in the environment. Many variables were introduced into the experiment. These variables consisted of four different hair subjects, two different types of hair roots, four environments, and use of a new hair at each collection point. Four hair subjects, as opposed to one, were used to add diversity to the study to determine if any major differences would occur between subjects. The anagen and telogen hair roots were used due to their differing degrees of keratinization. Telogen roots, due to their complete keratinization, served as controls, were more resistant to factors in the environment that could give rise to root degradation. Anagen roots, consisting of varying degrees of keratinization (dependent on their place in the growth cycle) were more susceptible to changes in root morphology, and degradation. Four experimental environments were used to simulate conditions that may be encountered at a crime scene.

Hair roots were morphologically changed as a result of being exposed to soil burial and water immersion. Neither environment caused more severe changes than the other. Hairs from some subjects developed patterns of degradation (banding, fraying, darkening, etc.) earlier in soil, others in water. While roots from both anagen and telogen phases developed patterns of morphological change, those of the anagen phase were more drastically altered. With time, the patterns that appeared in anagen roots became more exaggerated, sometimes such that the proximal end was completely obliterated. In contrast, the telogen roots were resistant to complete adulteration by the environments. Some telogen roots appeared unscathed, while others only had mild patterns of fraying or darkening, as compared to anagen roots. The difference in degrees of degradation between anagen and telogen hairs was pronounced and consistent. This suggested the idea that anagen hairs were more vulnerable to changes by the environment.

Anagen hairs were altered to a greater degree because they are not as completely keratinized as telogen hairs are. Repeated patterns of change occurred at the root end, suggesting that a similar type of structural alteration was taking place. There was inter- and intra-subject variation as to the frequency of certain patterns. However, all subjects exhibited similar patterns, which were darkening of the root tip, erosion of the root structure, frequently resulting in a pointed or brushy tip, and black banding at various distances from the proximal tip of the hair.

The banding and brush-like tip patterns that occurred in hair bore a striking resemblance to published images of postmortem root changes, specifically banding, both distal and proximal to the root (Linch et al. 1998; Linch and Prahlow 2001). Linch et al. (1998) performed an experiment to determine the effects of incubating hairs in Proteinase K. They reported the appearance of a "dead man's" root after this incubation. The image of a hair root from this experiment showed a hair's proximal end with a pointed tip and dark banding. These changes would be characterized as proximal root banding by Linch and Prahlow (2001). This was interesting because conditions in

this study--hairs collected from living subjects--produced root patterns that are currently associated with those from postmortem samples.

Petraco et al. (1988) reported that postmortem root bands are clusters of air pockets that appear opaque under transmitted light. When sliced open longitudinally and examined with a scanning electron microscope, parallel, elongated spaces were observed in the hair shaft. It was also mentioned that with light microscopy, when these bands are examined with incident, or overhead, light, they appear as bright portions in the hair shaft. Hairs from this experiment were examined with incident light microscopy and this brightening effect was evident. See Figure 22, which features photomicrographs of banded hairs under both transmitted and incident illumination.

This experiment was important in establishing that environments, particularly soil and water, do alter the morphology of hair roots. The hypothesis that anagen hairs would be more susceptible to changes was supported. Lastly, some of the patterns that arose from environmental exposure appeared similar to those encountered in postmortem samples, which has very important forensic implications for interpretation of hair evidence. While these patterns, arising from environmental exposure, could be confused for postmortem root bands, it is important to point out that the root bands that appeared in this study were very proximal to the anagen root end. This is not an accepted criterion for postmortem root banding. Examiners such as De Forest and Petraco et al. (1988) pointed out that bands appear more distal to the anagen root end, usually about 0.5 mm from the end, as opposed to bands appearing as distances of 0.05 to 0.3 mm from the root end, as seen in results of this study. This is also supported in published images of postmortem root banding (Tafaro 2000).

Problems that were encountered in this experiment are detailed below. Due to certain procedural conditions, the progression of changes in the hairs could not be assessed. Different hairs were collected at each time point, and an initial examination of each hair was impossible due

to the mounting protocol. Permanent mounts of hairs were made at each examination/collection, thus consuming the hair. Another problem that was encountered was that dark hairs of some of the subjects obscured certain changes. Darkening or banding was noted in some of these hairs, yet it was difficult to see. These two obstacles were resolved in the following experiments. Temporary, instead of permanent microscopical mounts were made to facilitate repeated examination at each time point. The subject that was used in the subsequent experiments had light hair that made visibility of certain degradation patterns more apparent.

Experiment #2:

Successive Root Morphology Changes Over Time Caused by Environmental Exposure

In this experiment, conditions were changed to accommodate some of the limitations of the first experiment, as explained above. The main focus of this portion of the study was to examine the progression of changes that occurred in the hair roots with increasing time spent in the environment. Both soil and water induced similar patterns of changes in hair. However, the hairs in the soil, once their proximal ends exhibited advanced changes, degradation at the proximal end seemed to slow. However, after seven months of exposure time, some hairs were found to be "chewed" from the tape mount by something in the soil, were thus irretrievable, and unable to be examined. This was not a problem in water; however, because hairs could be retrieved from the environmental tape mounts for the duration of the study (six to seven months). Continued collection and examination in these hairs revealed that hair degradation did not seem to slow, but continued to occur with time. One specific pattern that was unique to water-exposed hairs was the repeated appearances of several elongated black streaks, parallel to the length of the hair, clustered near the root end. It has been reported by Kaszynski (1985) that pre-keratin, which is present at the growing hair proximal end and possesses no disulfide bonds, swells moderately in

distilled water. This swelling could be occurring in the water-exposed hair samples, giving rise to air spaces in the shaft which would manifest itself as black streaks under transmitted light microscopy.

Experiment #3:

Comparison of Sterile and Nonsterile Hairs in Sterile and Nonsterile Environments

It was established in the first two sets of experiments that hair roots were being changed due to environmental exposure. However, the cause of these changes was unknown. This experiment introduced autoclaving into the preparation of hair samples and environments to see if root changes, i.e., degradation, were slowed in sterilized samples, due to an abolition of microorganisms from the environment. Another goal of the experiment was to try and determine if microbial sources responsible for degradation originated from the hair, the environment, or a combination of both.

Autoclaving did slow down the occurrence of degradation patterns and morphological changes in hair roots. All hair samples possessed some sort of change in their root morphology, yet those hairs that had been autoclaved exhibited changes at a later time as compared to un-autoclaved samples. It was difficult to tell if changes in hair originated from microbial activity from the hair or from the environment.

An analysis of the effects that autoclaving has on hair root structure, was performed. Hairs were microscopically examined and photomicrographed before and after sterilization and their morphologies were compared each time. Results in 13 hairs of either anagen or telogen origin showed that autoclaving did not change or alter the structure of the root (data not shown).

One problem with this experiment was that autoclaved environments were not kept sterile due to repeated unsealing of environments with each time point examination. This could have

introduced microorganisms, which produced the observed changes in the hair roots. This was addressed in the next experiment.

Experiment #4:

Comparison of Anagen and Telogen Hairs Housed in Sealed, Sterile Environments and in Open, Nonsterile Environments

This experiment examined the results of keeping sterile environments sealed, completely inhibiting the invasion of microorganisms into the system. Due to the results of the experiment, it appears that soil, when sterilized, did not produce changes in hair root structure. Sterile water, on the other hand, appeared to produce root changes. Both nonsterile soil and water produced changes. This suggested that there was another factor in water altering the root morphology, such as the aforementioned swelling phenomenon.

Experiment #5:

Repeated Placement and Removal of Hairs through Examination Does Not Create Artificial Changes in Root Morphology

This brief experiment proved that hair root morphological changes occurred as a result of environmental exposure, not mechanical processing of the hairs by the examiner. Repeated examination of hairs was postulated to be a cause of degradation patterns seen in the root ends of hairs. However, the results of this experiment proved that this was not the case. Moreover, the degradation patterns seen in this experiment appeared similar to those hairs that were examined repeatedly.

7. Conclusions and Future Ideas

This study evaluated the changes in hair roots, both anagen and telogen, when exposed to soil burial and water immersion. Reproducible results were obtained which suggested that a similar route of structural root alteration was occurring. In hairs possessing anagen roots, alteration in root morphology was highly advanced. This was due to a lack of structural integrity inherent in the incompletely keratinized proximal tip. In contrast, while hairs of the telogen phase did exhibit root changes, they were very mild in comparison to anagen root changes. Anagen roots became highly altered with patterns of end darkening, banding, fraying, and sometimes complete obliteration of the root. Telogen roots remained recognizable, despite minor alteration, such as darkened or frayed root bulb edges, by environmental exposure. This implies that, at the root bulb, there may be unkeratinized material adhering to the root that may be vulnerable to changes.

It is could not be determined whether water or soil gave rise to a greater degree of change in hair root morphology. In this study, it appeared that, in most instances, each environment caused an equal degree of change in hair roots by the end of the incubation period. One exception was with experiment #4; sterile water produced changes in anagen hair roots, namely tip darkening, and sterile soil did not affect the hairs. This is an area that should be explored further. This knowledge could help elucidate the conditions that would give rise to more advanced degradation.

As far as determination of the causes of degradation or morphological change in hair roots, only one possible cause was explored in this study. This was the area of microorganismal attack. Sterility mainly halted, if not slowed the development of morphological changes in hair roots, implying that microbial factors were causing hair root changes. It is known that certain species of keratinolytic fungi are responsible for the degradation of keratin in hair shafts. However, the

microbes that may cause hair root degradation are unknown. Isolation of microbes from soil and water samples that are known to give rise to hair root degradation would be a valuable step in determination of certain bacterial and fungal species that thrive on the material in hair roots.

Other causes for hair root degradation were briefly mentioned earlier. One includes physical alteration from the environment, namely swelling from water. Others include chemical alteration. Choudry et al. (1983) explored the results of application of reducing agents to hair shafts to produce characteristic patterns of surface changes. One could experiment with differing pH levels in the water and soil to examine whether this would enhance or prevent hair root morphology changes.

Hair morphology is highly variable intra-personally, as discovered in this study. This could explain the varied levels of morphological change in a group of anagen or telogen roots from the same subject. Because hair follicles grow independently of each other, this suggests that root areas of hairs plucked from one's scalp possess varying degrees of keratinization. For example, one anagen hair may be in the late anagen phase, consisting of a higher degree of keratinization near the root, while another may be in mid-anagen phase where the root keratin levels are reduced in comparison. This would imply that the latter sample would exhibit more changes after environmental exposure due to its weaker integrity. A study exploring this topic should be done to elucidate the variability encountered in hair root morphology change. Something such as histological staining for keratin could be used to assess the levels and location of this protein at the proximal tip of roots from different growth phases.

Lastly, a survey of environmentally exposed hair roots and decomposed hair root samples utilizing light microscopical examination should be conducted to assess the morphological similarities between these two types of hair roots. In this study, some hairs produced patterns that could have been construed as postmortem root changes. One should bear in mind that banding

patterns in postmortem hair roots appear more distal to the anagen root end as opposed to the banding patterns in the environmentally exposed hairs, which appeared proximal to the anagen root end. This confusion could have major implications on the examination of and subsequent interpretation about hairs found at a crime scene. The consequences of mis-assigning a hair as belonging to a postmortem source when it has actually only been changed by the environment could affect the results of a forensic case with serious consequences.

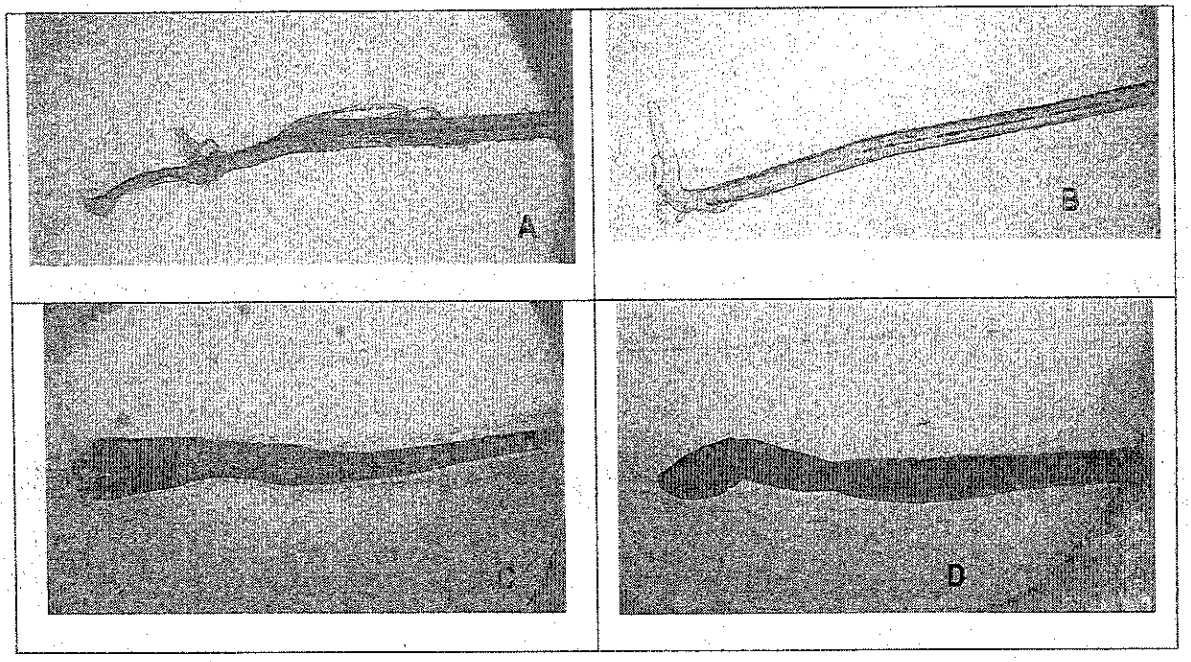

Figure 1: Images of anagen roots

Featured above are four different examples of anagen root morphology in different subjects. Images were collected in this study. Note highly variable morphology in each sample. Consistent within each root is the amorphous structure characteristic to anagen roots. Transmitted light microscopy at 100x magnification.

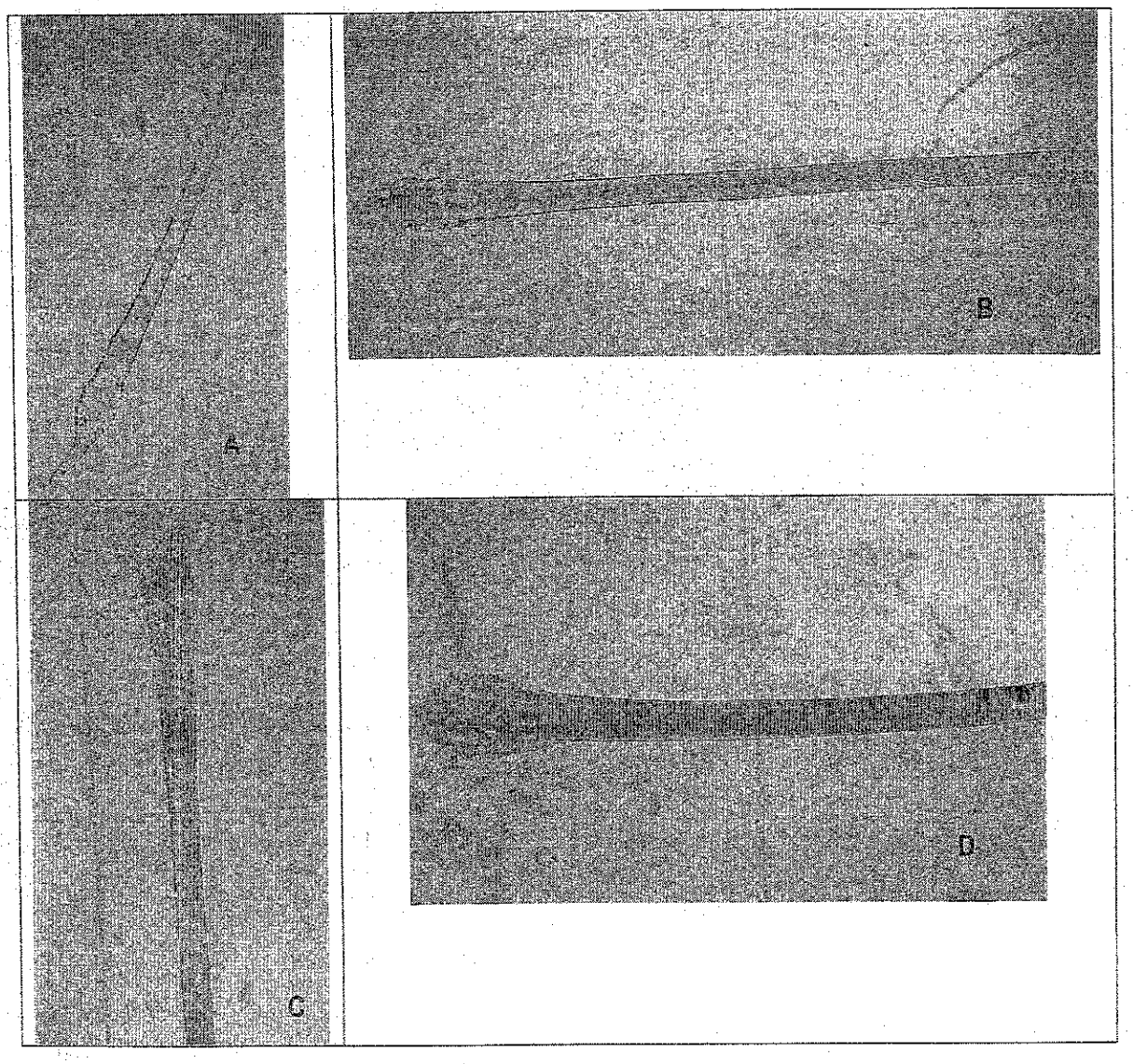

Figure 2: Images of telogen roots
These hairs were collected in the study. Although possessing inter-subject variation in appearance, all four examples possess the normal telogen root morphology. Note the rigid, bulbous structure of the root. Image A displays a telogen root with a germinal nipple. Transmitted light microscopy at 100x magnification.

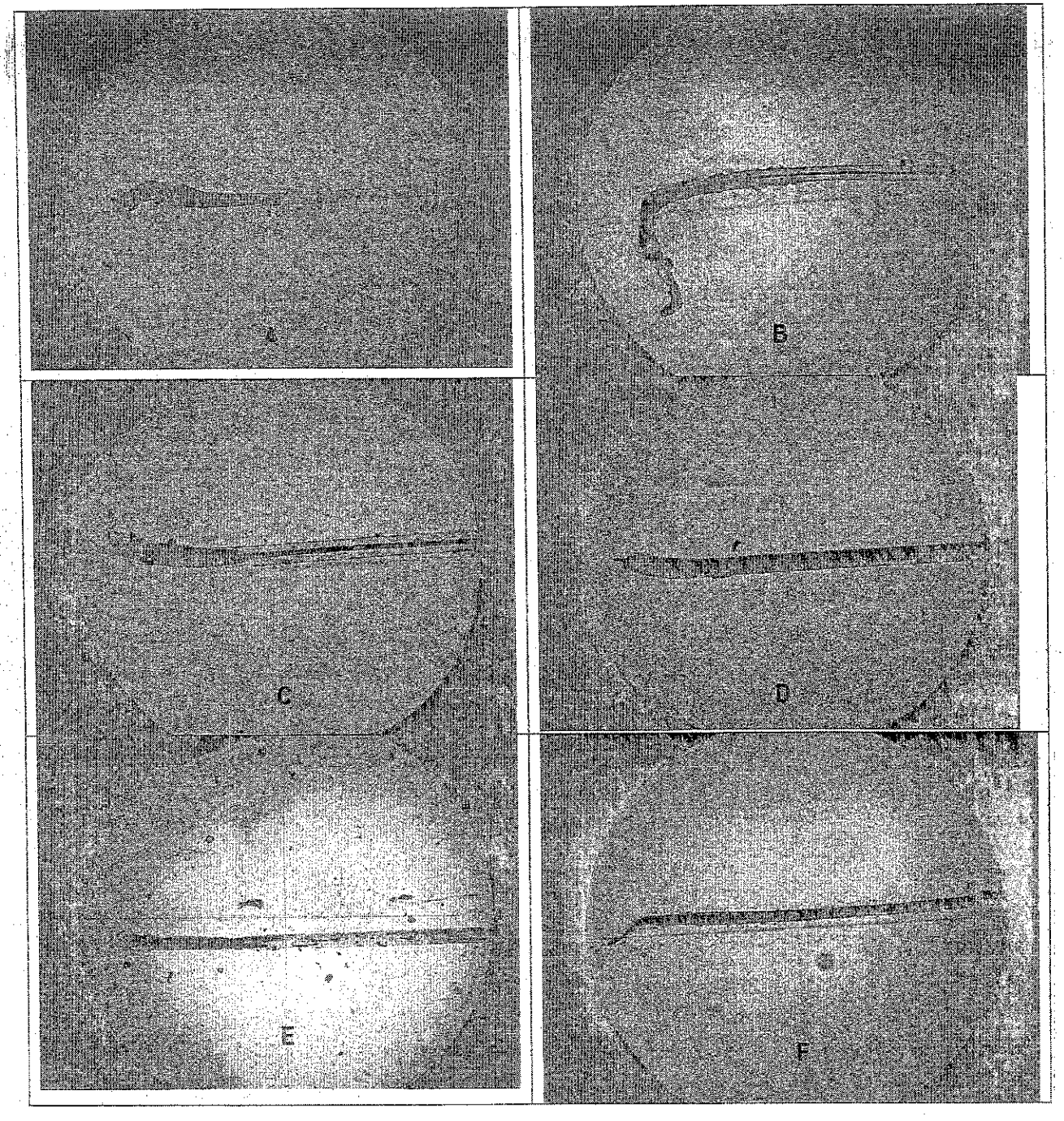

Figure 3: Experiment #1 Anagen hair roots after soil exposure

Patterns of degradation in anagen roots after various times in soil are shown with transmitted light microscopy at 100x magnification.

Left to right: A: Subject #4, banded root (6 day exposure), B: Subject #2, darkened shriveled tip (1 day exposure), C: Subject #2 brushy, banded tip (12 day exposure), D: Subject #3, brushy tip (6 day exposure), E: Subject #4 darkened, shriveled tip, with banded area (60 day exposure), F: Subject #1 pointy tip with darkening (30 day exposure).

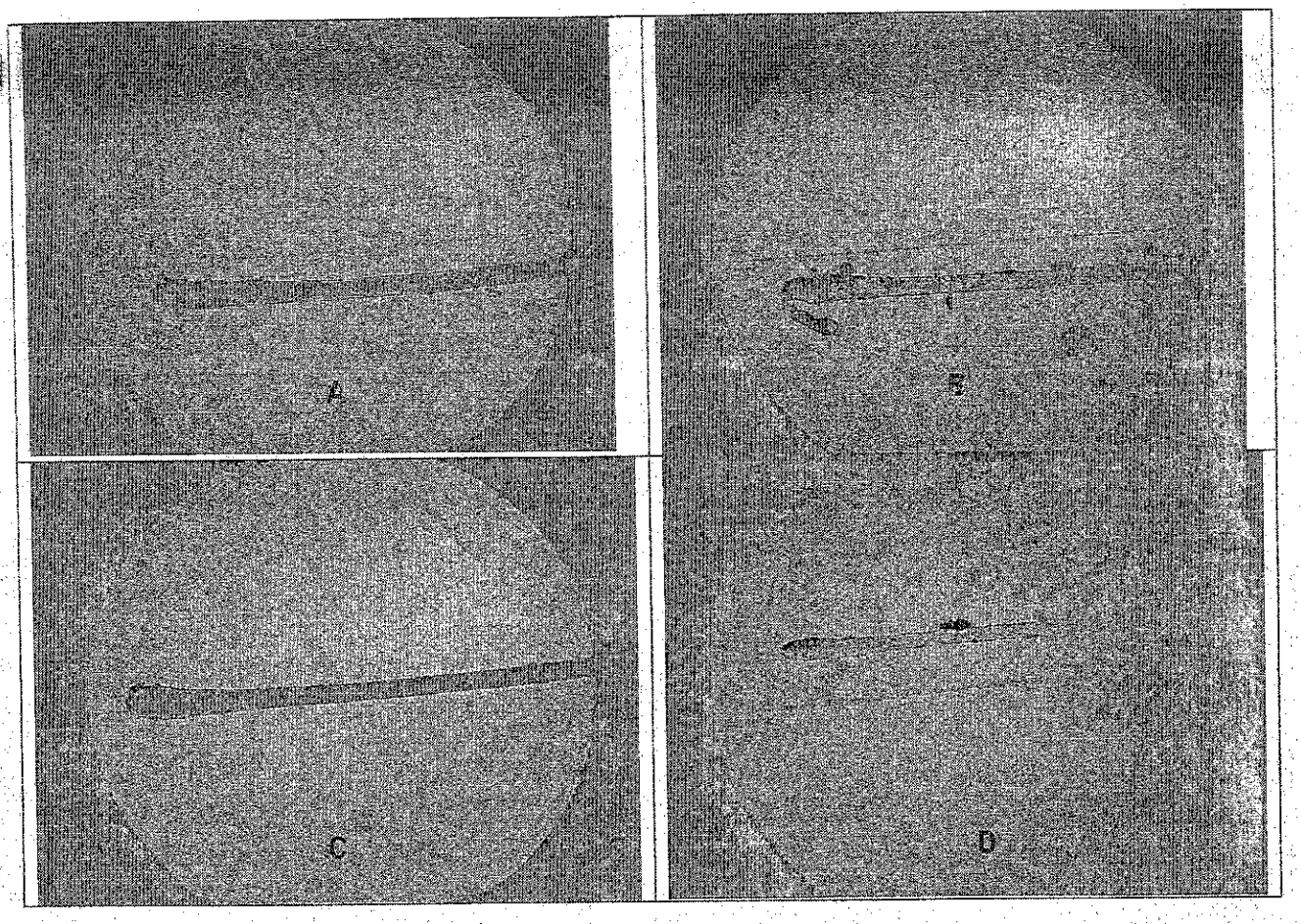

Figure 4: Experiment #1 Telogen hair roots after soil exposure
Various telogen hairs exposed to soil are shown with transmitted light microscopy at 100x magnification.
Left to right: A: Subject #2 exhibited darkening of bulb (6 day exposure), B: Subject #1 exhibiting tip darkening and adherence of particulate matter (30 day exposure), C: Subject #3 exhibiting slight bulb darkening (2 day exposure), and D: Subject #4 exhibiting tip darkening and slight erosion of bulb (9 day exposure).

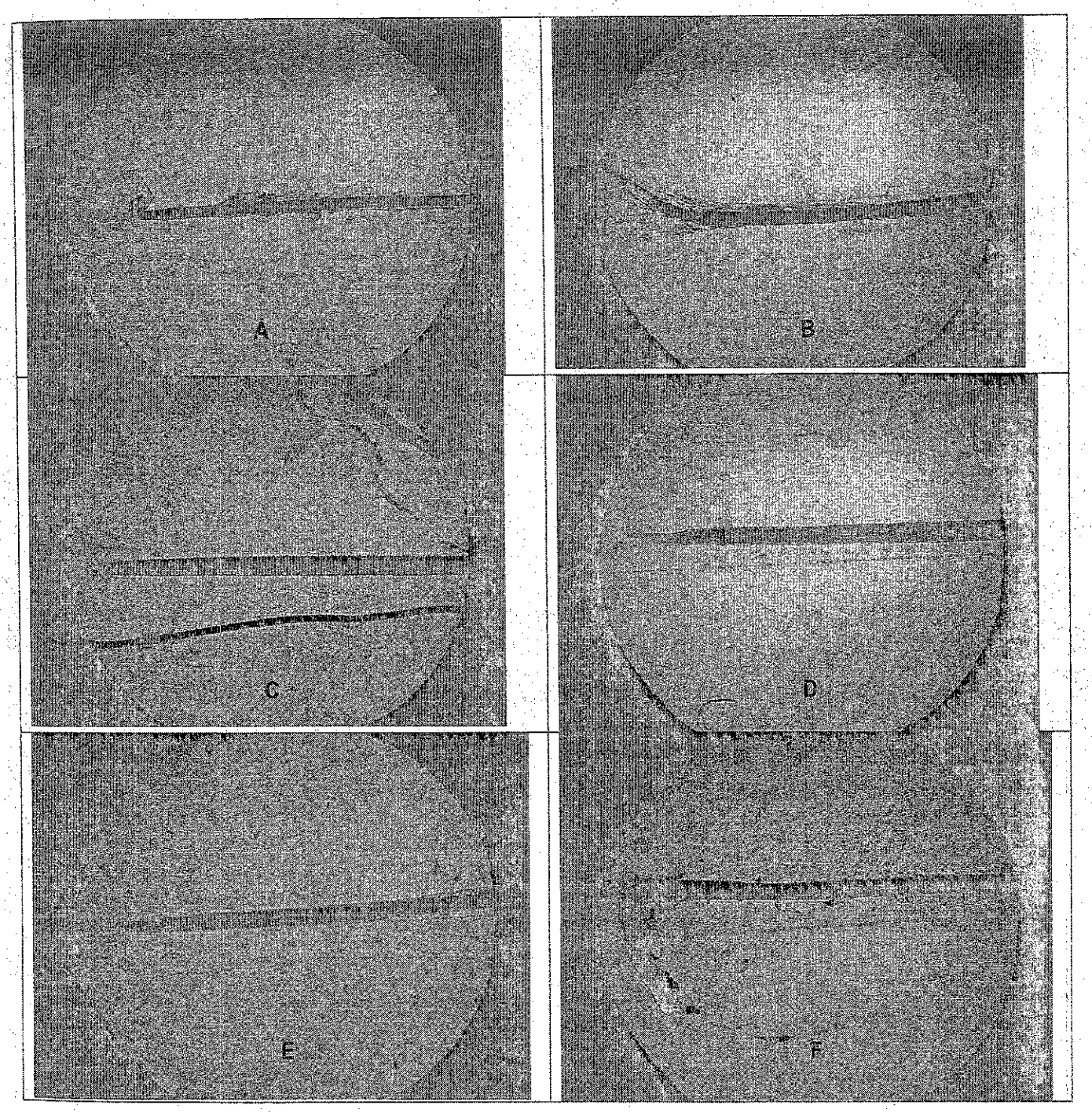

Figure 5: Experiment #1 Anagen hair roots after water immersion

From left to right: A: Subject #1 shriveled, darkened root with particulate matter adhering (9 day exposure), B: Subject #2 with abundantly attached green algal growth and a darkened tip (60 day exposure), C: Subject #3 Completely eroded root structure, darkening present, adherence of flaggellar organism (30 day exposure), D: Subject #4 banded root with elongated streaks of black, shriveled tip (2 day exposure), E: Subject #4, large band proximal to tip, eroded root tip (30 day exposure), F: Subject #1 shriveled, darkened root, with algal covering (4 day exposure). Transmitted light microscopy at 100x magnification.

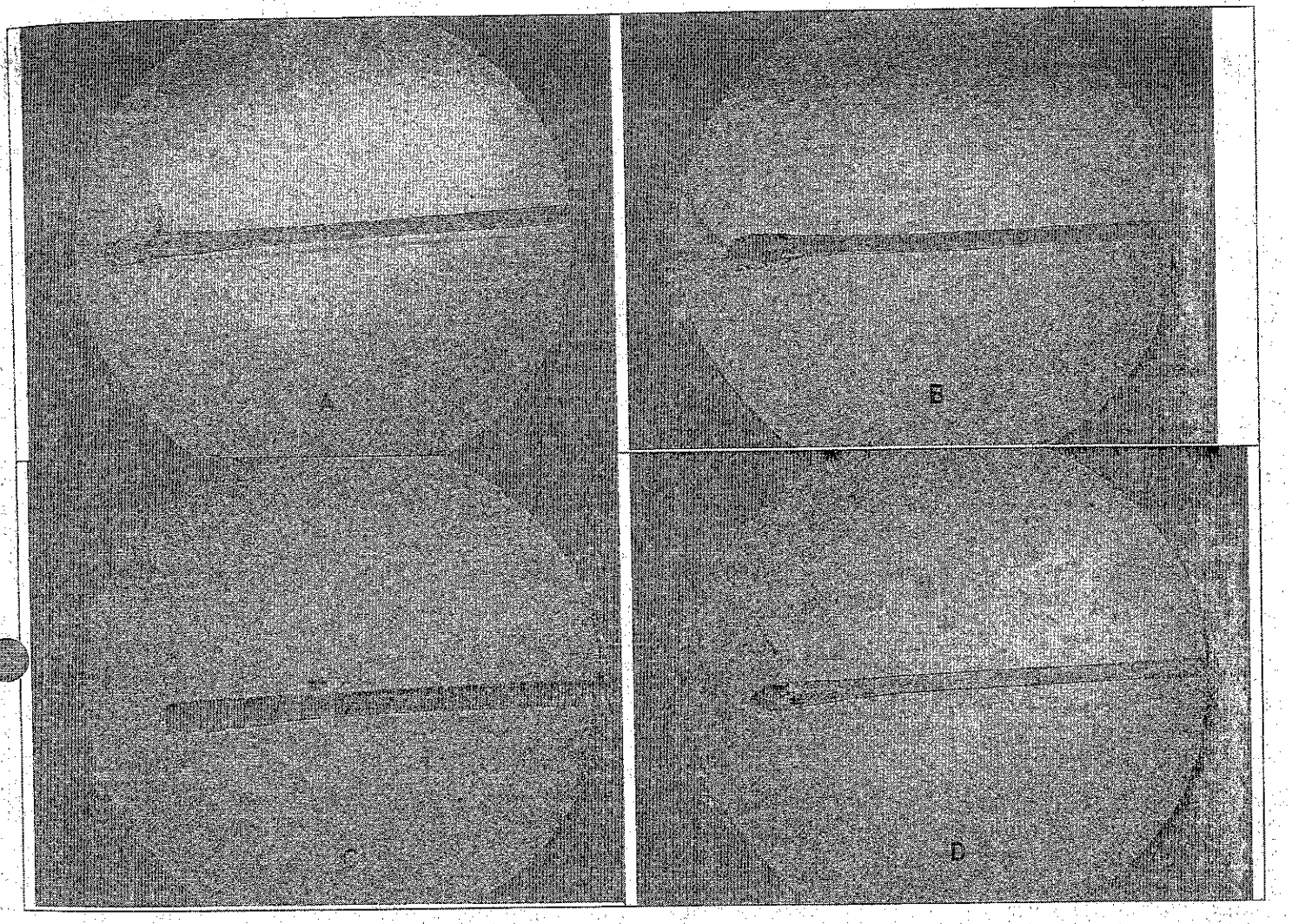

Figure 6: Experiment #1 Telogen roots after water immersion

From left to right: A: Subject #1 exhibiting adherence of microorganisms at the proximal end and shaft of hair (2 day exposure), B: Subject #2 possessing darkened tip with microorganisms attached (6 day exposure), C: Subject #3 with darkened bulb (12 day exposure), and D: Subject #4 with green microorganism attached at darkened bulb (30 day exposure). Transmitted light microscopy at 100x magnification.

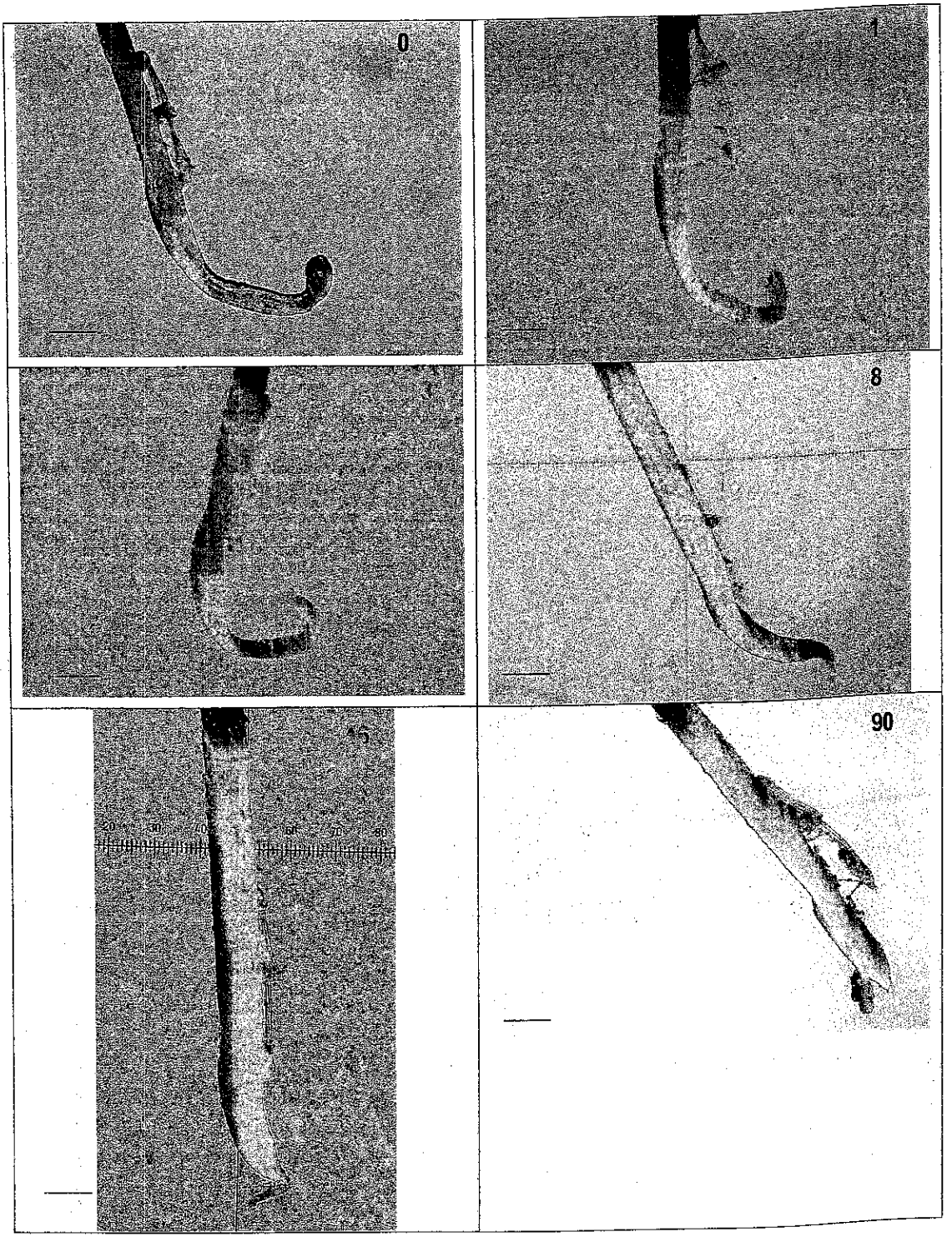

Figure 7: Experiment #2 Anagen hair exposed to soil for three months

Photomicrographs at 100x magnification. Black line in lower right corner equal to 100 μM. From left to right, exposure times in days are as follows, 0, 1, 3, 8, 16, and 90, as indicated in upper right corner of image. Note trend of shriveling, erosion of root structure to yield a pointy tip, and further erosion, which has reduced the length of the proximal tip.

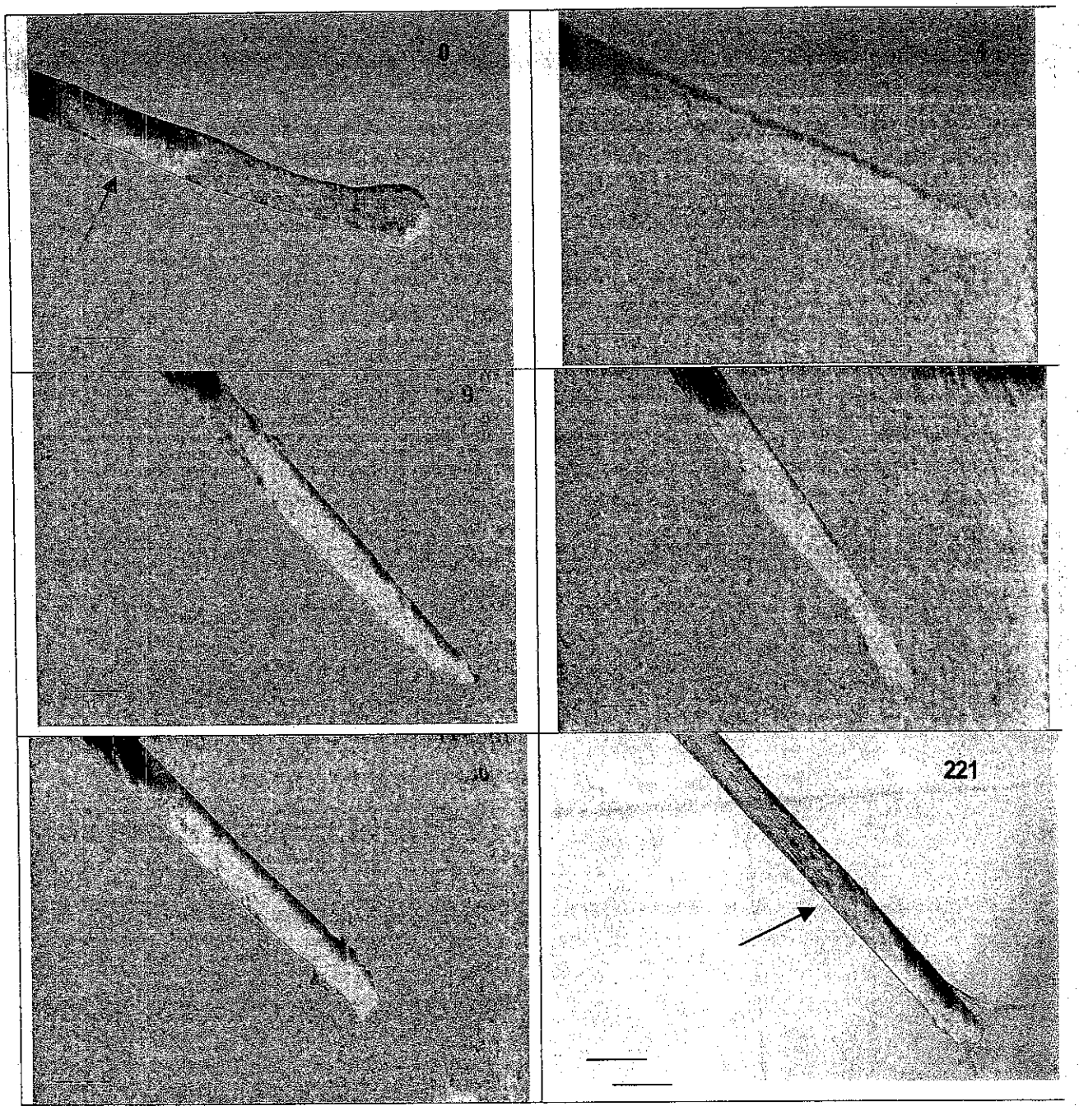

Figure 9: Experiment #2. Anagen hair exposed to water for 7 months

Photomicrographs at 100x magnification. Black bar equals 100 μM. From left to right, exposure times in days are 0, 4, 9, 17, 30, and 221. Black marking on hair (0 day exposure) (see arrow in upper left image) was placed on hair as a reference, this mark chipped off the hair over time. Note the progression of changes in this hair, erosion of root to yield a pointy tip, followed by appearance of darkening and elongated black streaks (see arrow in lower right image). Length of root tip appears to have been shortened over time.

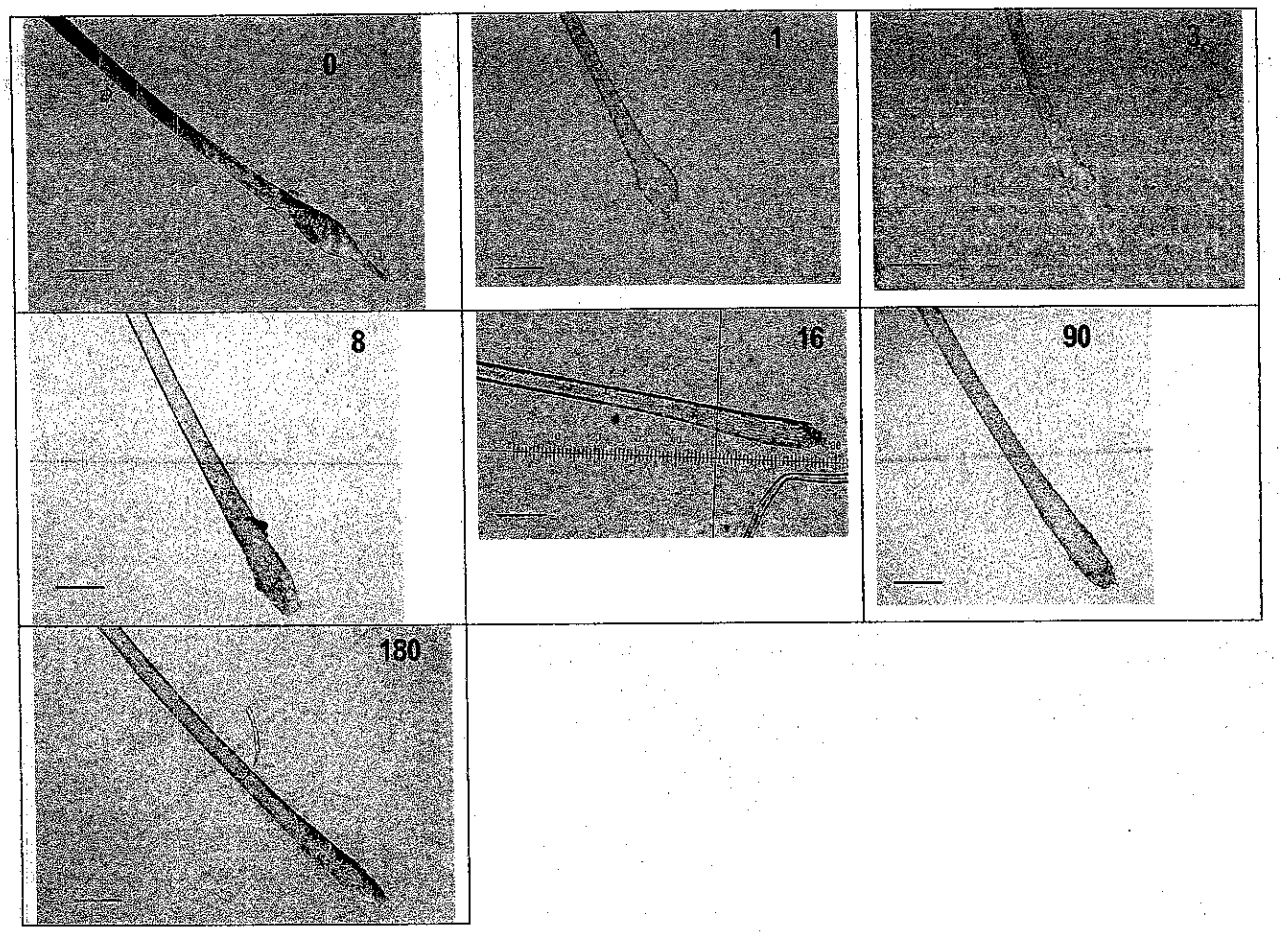

Figure 10: Experiment #2: Telogen hair exposed to water for 6 months

Photomicrographs at 100x magnification. Black bars equal 100 μM. From left to right, exposure times in days are 0, 1, 3, 8, 16, 90, and 180. Image in upper left corner (0 days) possesses red color on shaft from dyeing. This color faded over time. Note presence of germinal nipple in beginning images. This germinal nipple disappears with time, tip has also frayed and darkened at bulb.

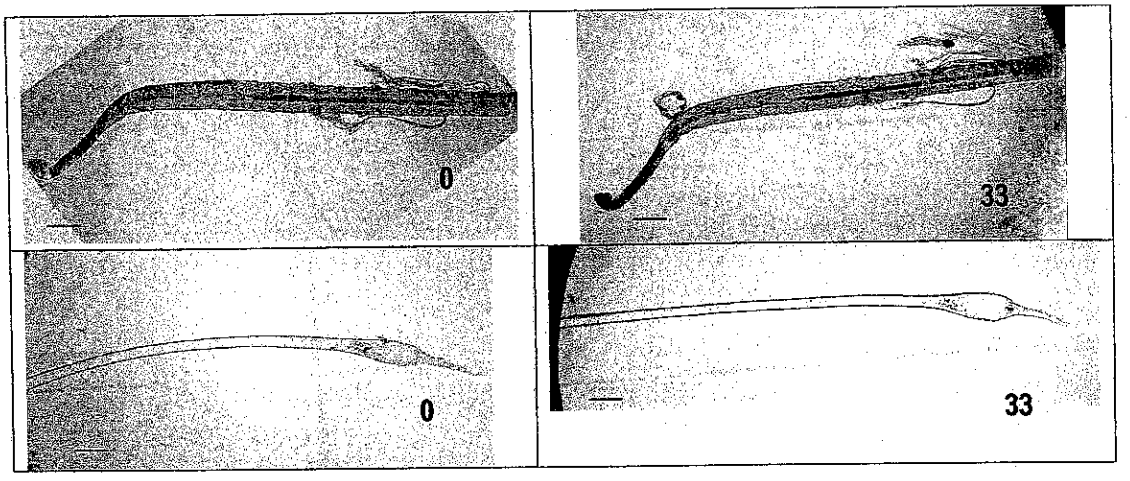

Figure 11: Experiment #3 Anagen and Telogen Hair Roots after 1 month, 3 day ambient exposure
Photomicrographs at 100x magnification. Black bars equal 100 μM. Top row features anagen hairs, bottom row features telogen hairs. Note no change in root morphology or appearance in either root type.

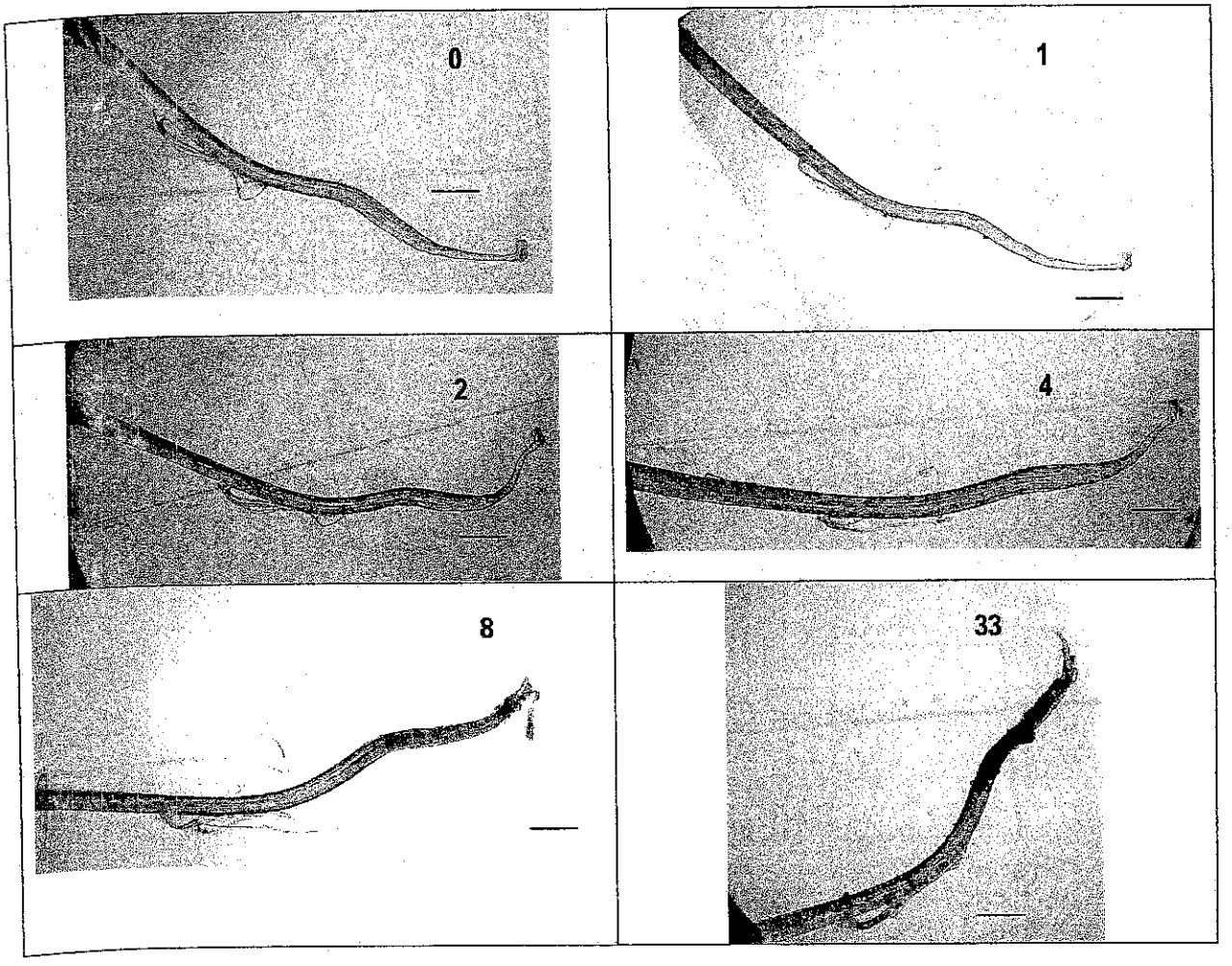

Figure 12a: *Autoclaved* anagen hair stored in *autoclaved* soil for 1month, 3 days

Photomicrographs at 100x magnification. Black bar is equal to 100 μM. From left to right, exposure times in days are 0, 1, 2, 4, 8, 33, as indicated in the upper right corner of each image. Note initial shriveling of root tip at 4 days which progresses to an eroded, pointy tip possessing a dark region at 8 and 33 days. This set of images is contrasted with that of Figure 12b. Note that onset of changes appears later than in hair featured in Figure 12b.

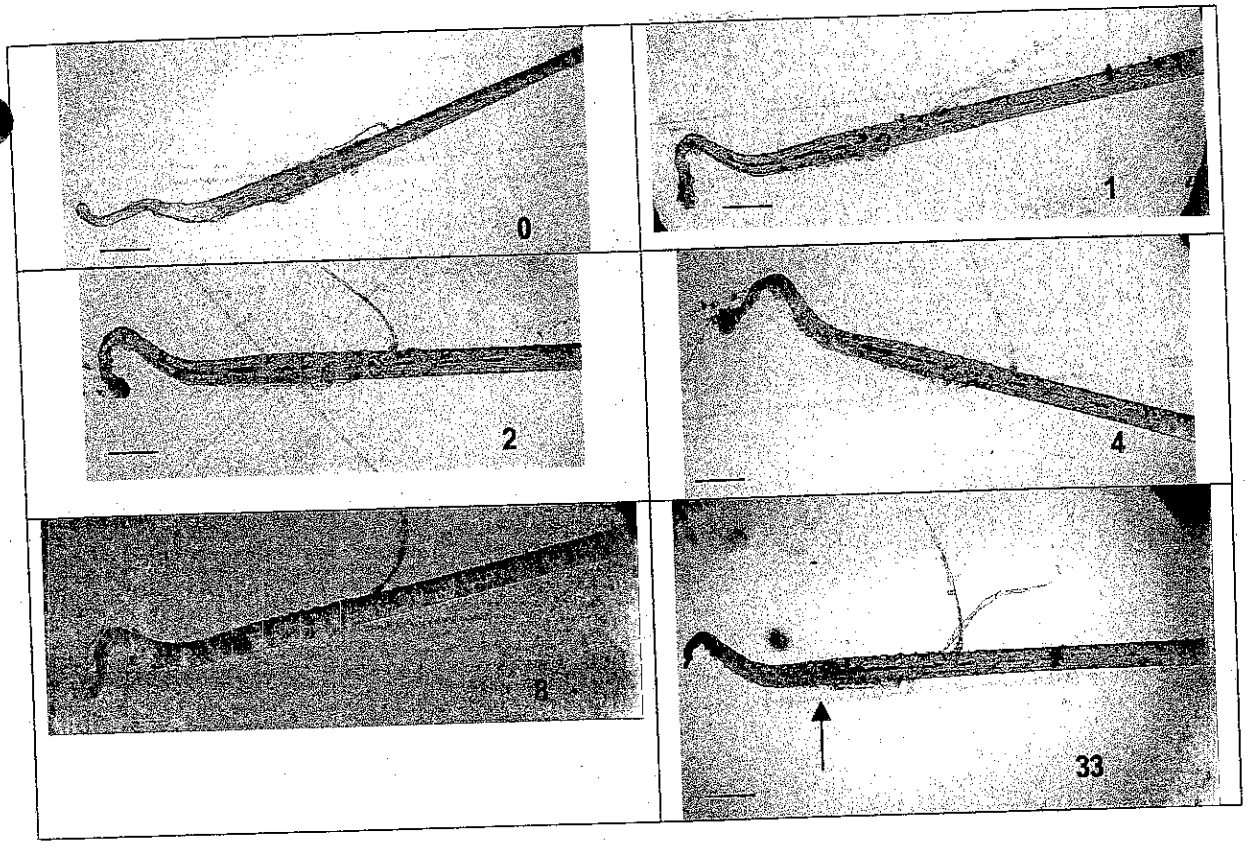

Figure 12b: Un-*Autoclaved* anagen hair stored in *autoclaved* soil for 1month, 3 days

Photomicrographs at 100x magnification. Black bar is equal to 100 μM. From left to right, exposure times in days are 0, 1, 2, 4, 8, 33, as indicated in lower right corner of image. Note initial bending of root tip at 1 day which progresses to a shriveled, eroded, pointy tip possessing a dark region at 33 days, see arrow in lower right image. This set of images is compared with that of Figure 12a. Note onset of changes starts earlier in this hair as compared to the hair in Figure 12a.

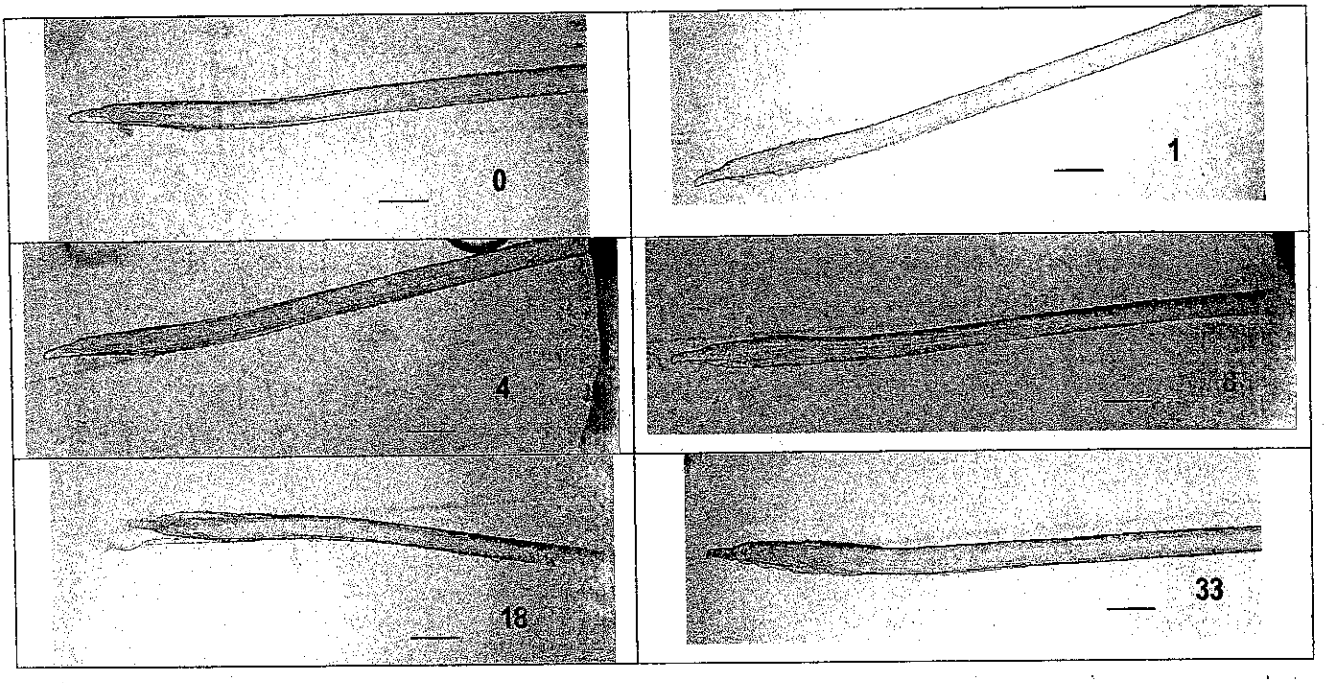

Figure 13a: *Autoclaved* anagen hair stored in *autoclaved* water for 1month, 3 days

Photomicrographs at 100x magnification. Black bar is equal to 100 μM. From left to right, exposure times in days are 0, 1, 2, 8, 18, 33, as indicated in lower right corner of image. Note minimal changes in hair root. Obvious morphological changes such as slight darkening of proximal tip are not apparent until about 18 days. This set of images is contrasted with that of Figure 13b. Note late onset of changes in this hair as compared to the hair in Figure 13b.

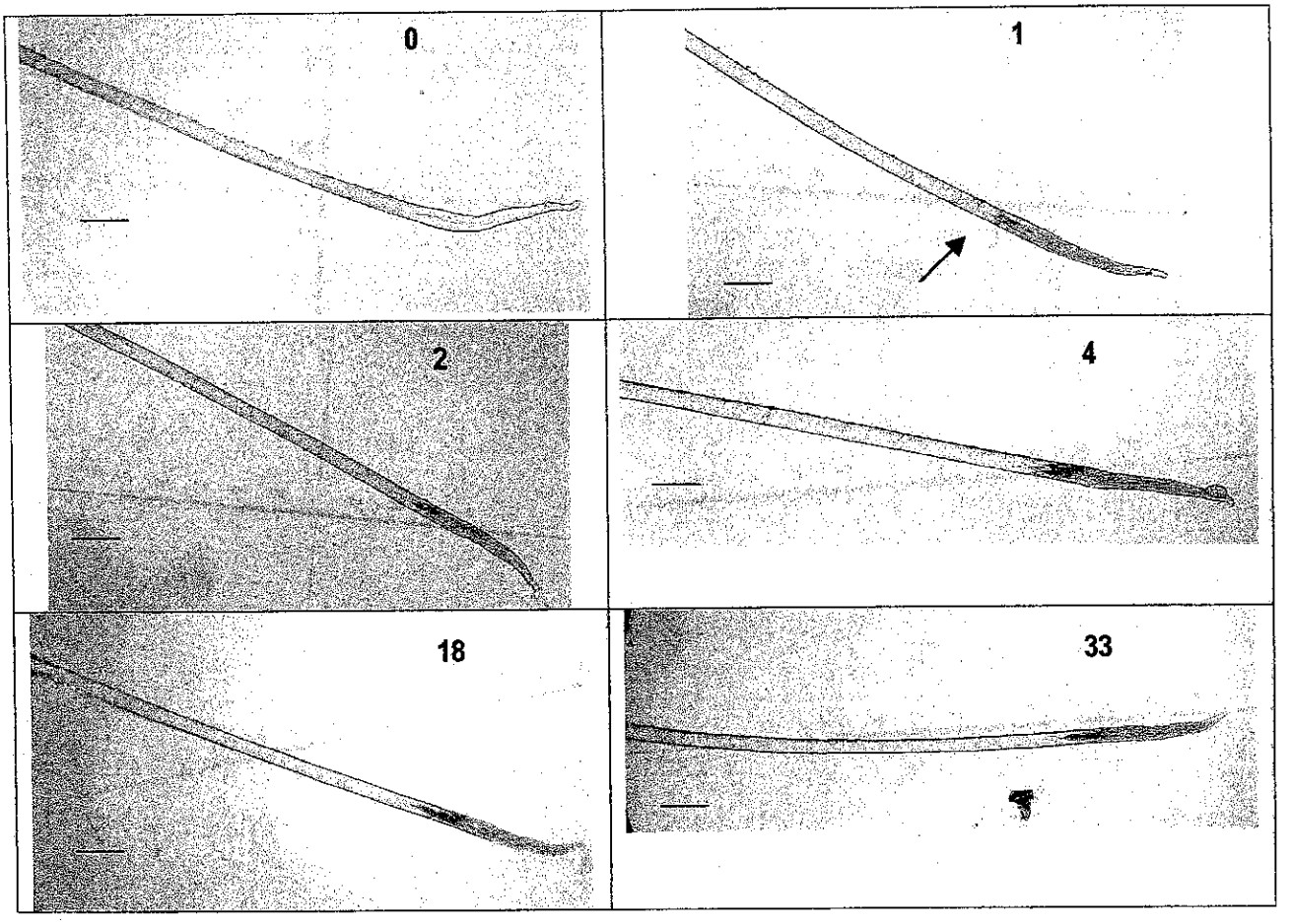

Figure 13b: Un-*Autoclaved* anagen hair stored in *autoclaved* water for 1month, 3 days

Photomicrographs at 100x magnification. Black bar is equal to 100 μM. From left to right, exposure times in days are 0, 1, 2, 8, 18, 33, as indicated in upper right corner of image. Note changes in hair root. Elongated black streaks begin to manifest themselves as early as one day (see arrow) and advance for the duration of the environmental exposure. This set of images is contrasted with that of Figure 13a. Note the earlier onset of changes in this hair as compared to the hair in Figure 13a.

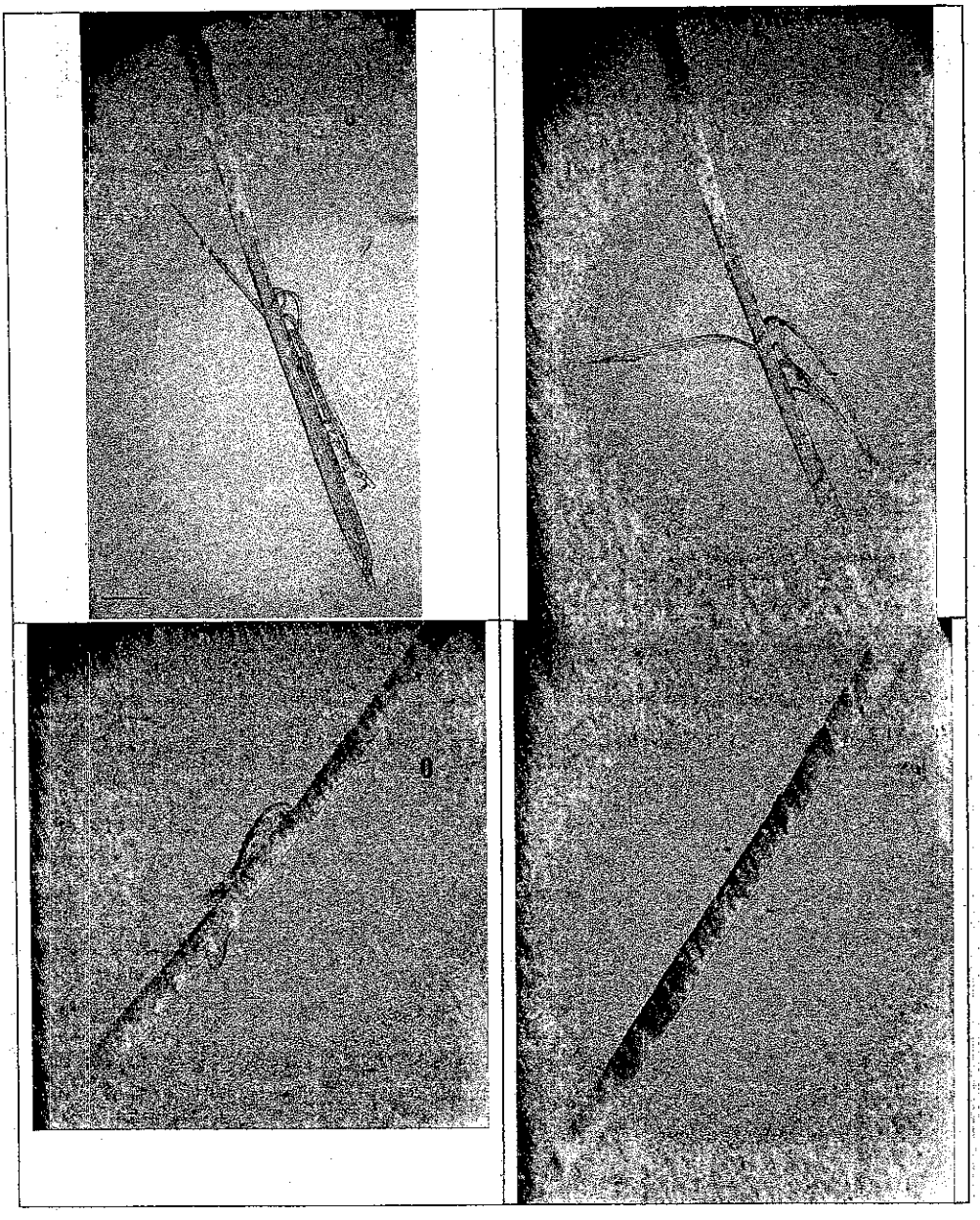

Figure 14: Experiment #4. Anagen hairs after 21-day exposure to sterile soil (top row) and nonsterile soil (bottom row)

Photomicrographs at 100x magnification. Black bar equals 100 μM. These hairs were exposed to the environment for 21 days. Images of left feature hair before environmental exposure; on right are hairs after 21 days of environmental exposure. Note the unchanged appearance of the hair in the top row. Note the changed appearance of the hair in the bottom row. In hair from bottom row, tendrils attached to shaft are missing and pointed tip has been eroded to a rounded tip.

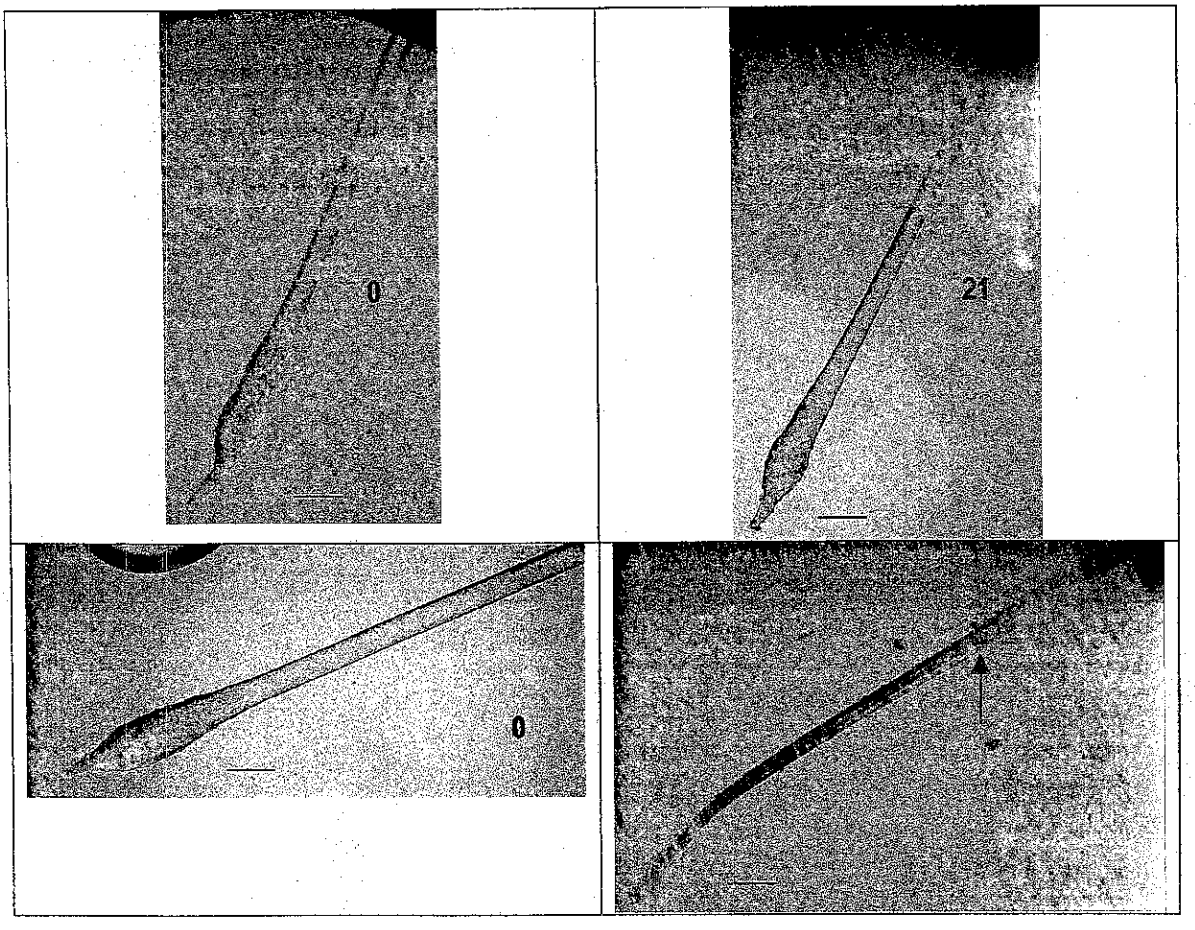

Figure 15: Experiment #4. Telogen hairs after 21-day exposure to sterile soil (top row) and nonsterile soil (bottom row)

Photomicrographs at 100x magnification. Black bar equals 100 μM. These hairs were exposed to the environment for 21 days. Images of left feature hair before environmental exposure; on right are hairs after 21 days of environmental exposure. Note the slightly changed appearance of the hair in the top row, it appears as a minimally shortened germinal nipple. Note the changed appearance of the hair in the bottom row. In hair from bottom row, root structure has not only been digested, but shaft has as well. Macroscopic digestion of this hair was noted; the length of the proximal hair had shortened after soil exposure. Arrow in lower right image points to an example of fungal tunneling.

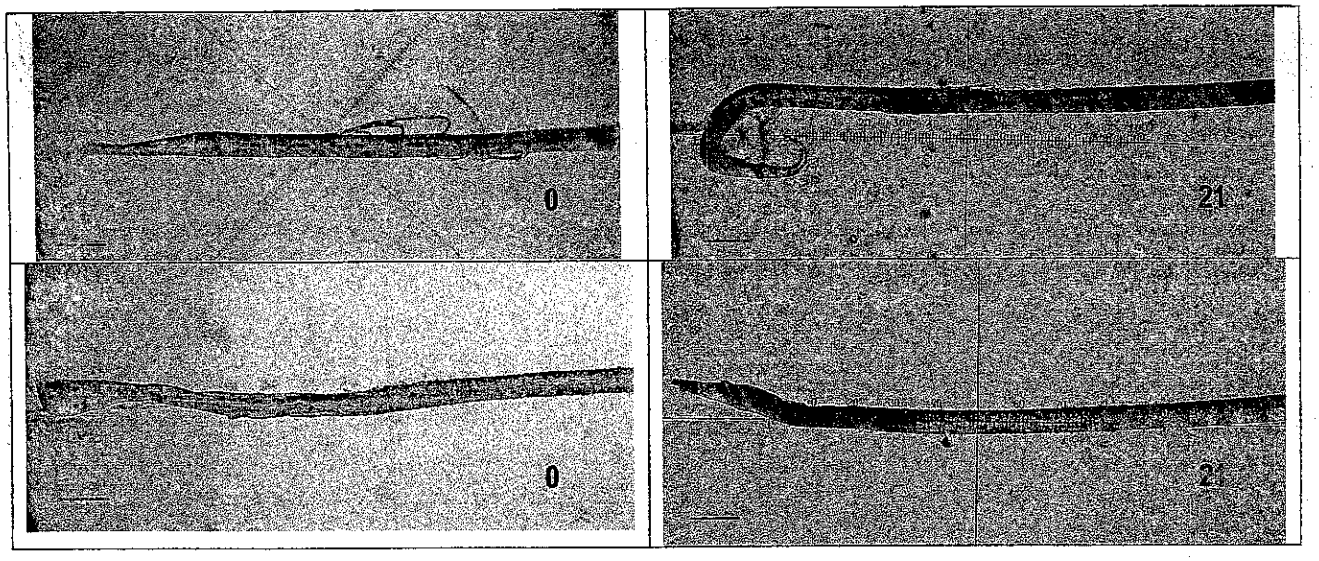

Figure 16: Experiment #4. Anagen hairs after 21-day exposure to sterile water (top row) and nonsterile water (bottom row)

Photomicrographs at 100x magnification. Black bar equals 100 μM. These hairs were exposed to the experimental environments for 21 days. Images of left feature hair before experimental environmental exposure; on right are hairs after 21 days of environmental exposure. Note that both sets of hair show change after experimental environmental exposure. The hair kept in the sterile water (top row) showed shriveling and curling of the root tip with slight darkening. The hair kept in the nonsterile water (bottom row) displayed erosion of the widened root to produce a narrow point. Darkening was also produced. This suggests that something other than microbial attack in water may be responsible for changing the morphology of anagen roots.

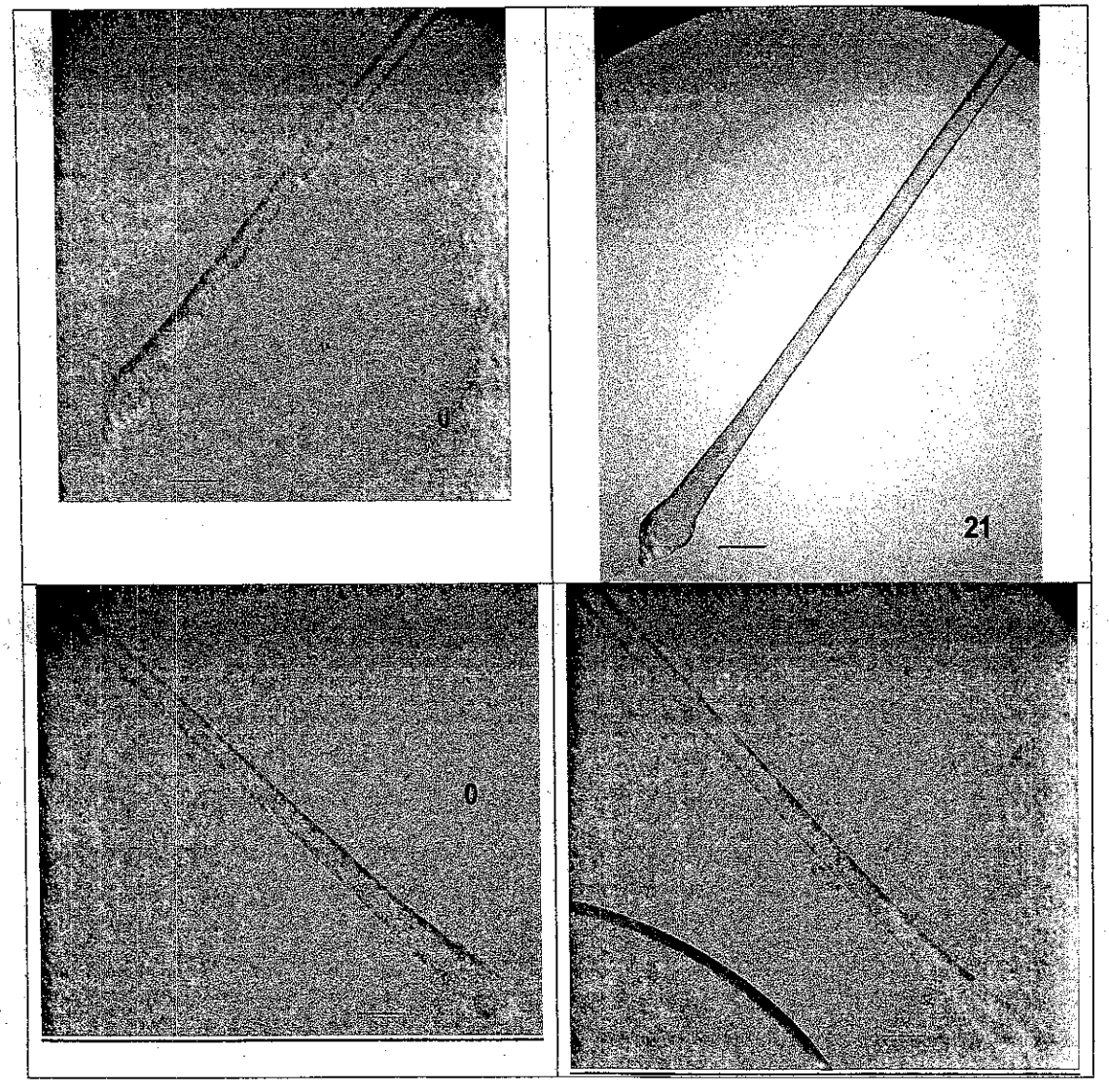

Figure 17: Experiment #4. Telogen hairs after 21-day exposure to sterile water (top row) and nonsterile water (bottom row)

Photomicrographs at 100x magnification. Black bar equals 100 μM. These hairs were exposed to the experimental environments for 21 days. Images of left feature hair before environmental exposure; on right are hairs after 21 days of environmental exposure. Note that both sets of hair show change after environmental exposure. The hair kept in the sterile water (top row) showed a germinal nipple that is missing after 21 days. The hair kept in the nonsterile water (bottom row) displayed darkening and fraying of the root bulb. This suggests that something other than microbial attack in water may be responsible for changing the morphology of telogen roots.

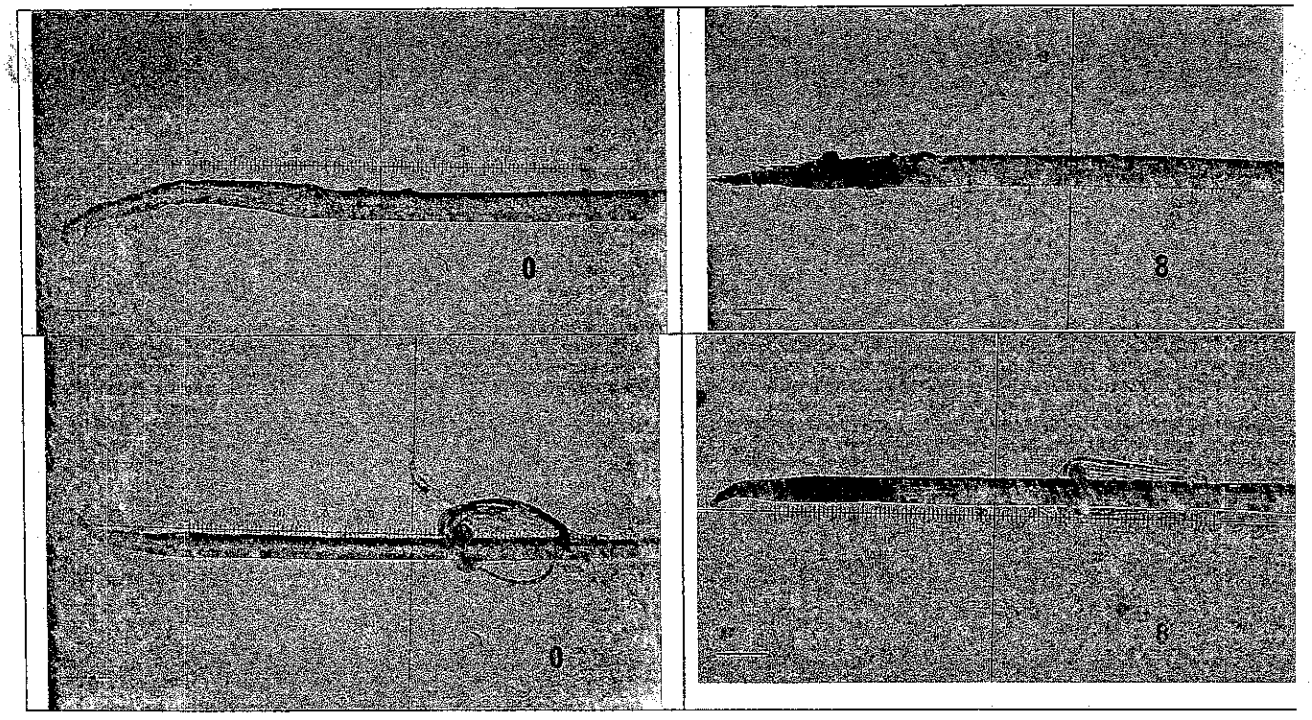

Figure 18: Experiment #5. Anagen hair roots after soil (top row) and water (bottom row) exposure
Photomicrographs at 100x magnification. Black bars are equal to 100 μM. The hairs were stored in soil and water for 8 days and examined once to minimize mechanical disturbance. Images on the left feature hair root morphology before environmental exposure; images on right feature the same hairs 8 days after exposure. Note darkening in both sets and erosion from soil, and shriveling from water. Because hair root morphology appears changed, it can be concluded that in past experiments, mechanical disturbance by the examiner did not create any visible background degradation or change in morphology. Changes in hair roots were solely caused by environmental exposure.

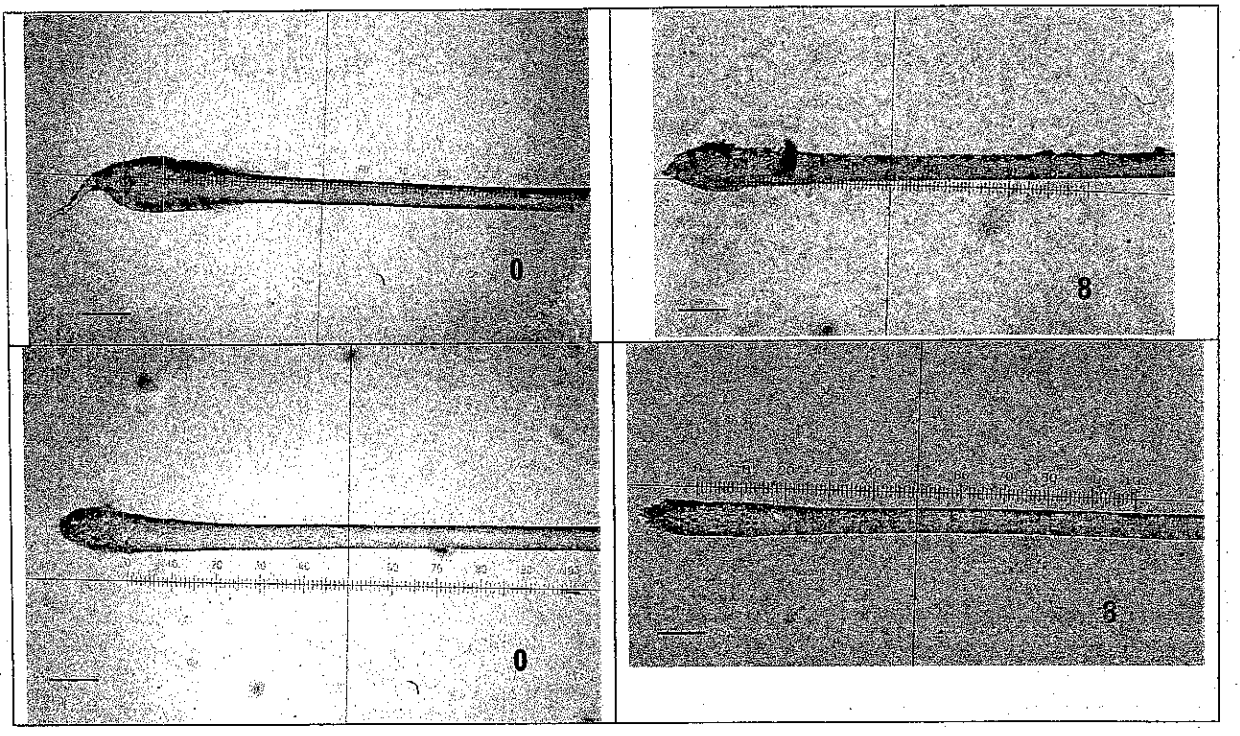

Figure 19: Experiment #5. Telogen hair roots after soil (top row) and water (bottom row) exposure
Photomicrographs at 100x magnification. Black bars are equal to 100 μM. The hairs were stored in soil and water for 8 days and examined once to minimize mechanical disturbance. Images on the left feature hair root morphology before environmental exposure; images on right feature the same hairs 8 days after exposure. In soil-exposed hair, note missing germinal nipple and tissue sheath around bulb in image on top right. In water-exposed hair, note darkening and fraying of bulb edges. Because hair root morphology appears changed, it can be concluded that in past experiments, mechanical disturbance by the examiner did not create any visible background degradation or change in morphology. Changes in hair roots were solely caused by environmental exposure.

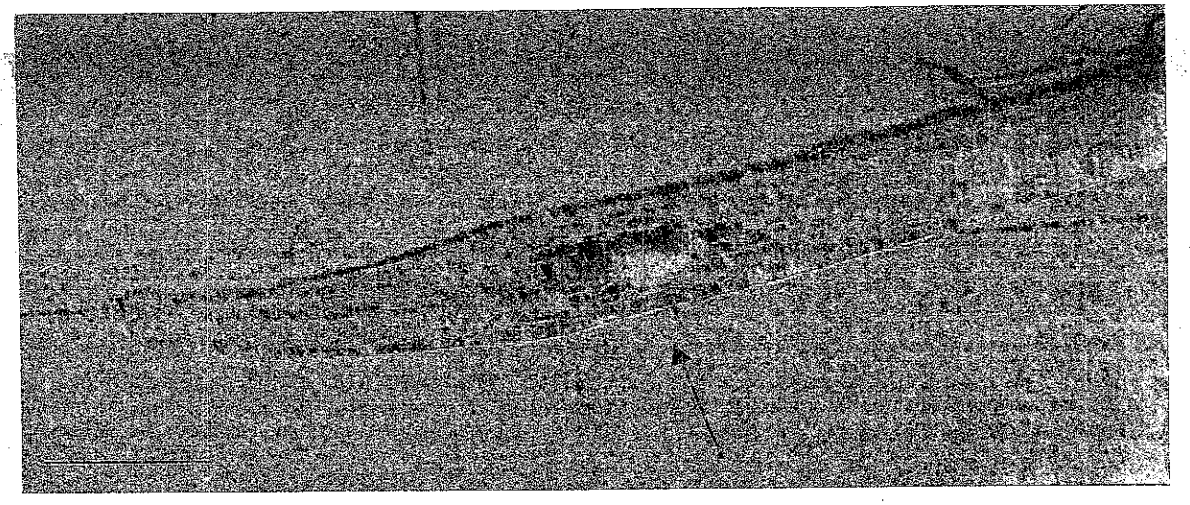

Figure 20: Elongated black streaks at the proximal root end

Photomicrograph at 200x magnification. Black bar equals 100 μM. Featured is an anagen hair that was exposed to un-autoclaved water for 18 days. This pattern has been described as vacuolization, or formation of air pockets. The cause is unknown, but it could be due to swelling of the pre-keratin after storage in water.

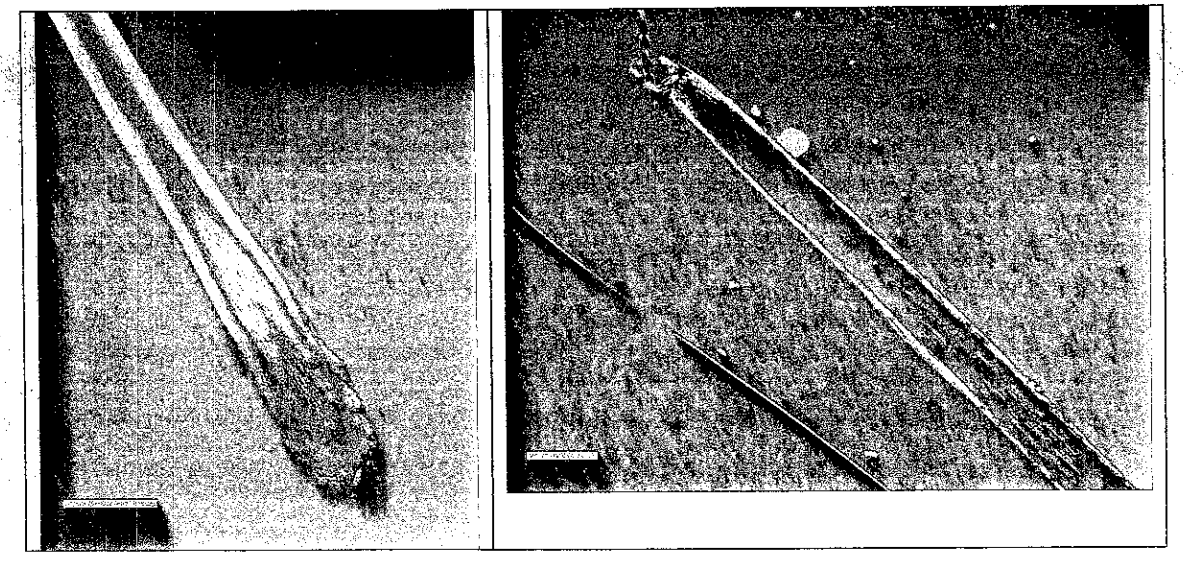

Figure 21: Anisotropy of hair.

Photomicrographs at 200x, 100x magnification, respectively. Yellow bar equals 100 μM. Hairs were examined under crossed polars, displaying the brilliant birefringence of keratin. A telogen hair is featured at left; an anagen hair is featured at right. Hairs were stored in water for 18 days and 2 months, respectively. Note the dark band and brushlike tip present in anagen hair root at right.

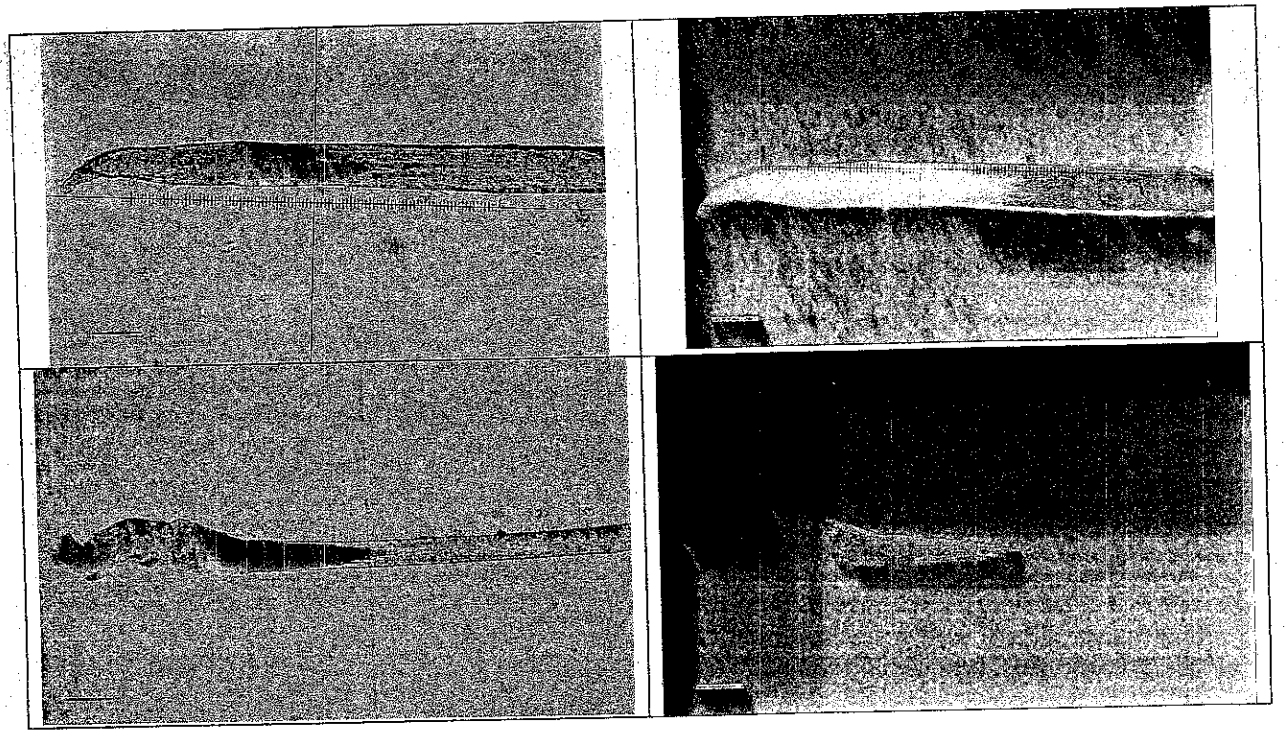

Figure 22: Images of darkening and banding under transmitted and incident illumination

Photomicrographs at 100x magnification. Black and green bars equal 100 μM. Images on left are illuminated with transmitted light; on right, images are illuminated with incident, or overhead light. These darkened or banded portions are air-filled spaces. The enclosed air scatters the transmitted light, making the area appear dark. Under incident light, these air-filled spaces appear bright. These images were collected during this study.

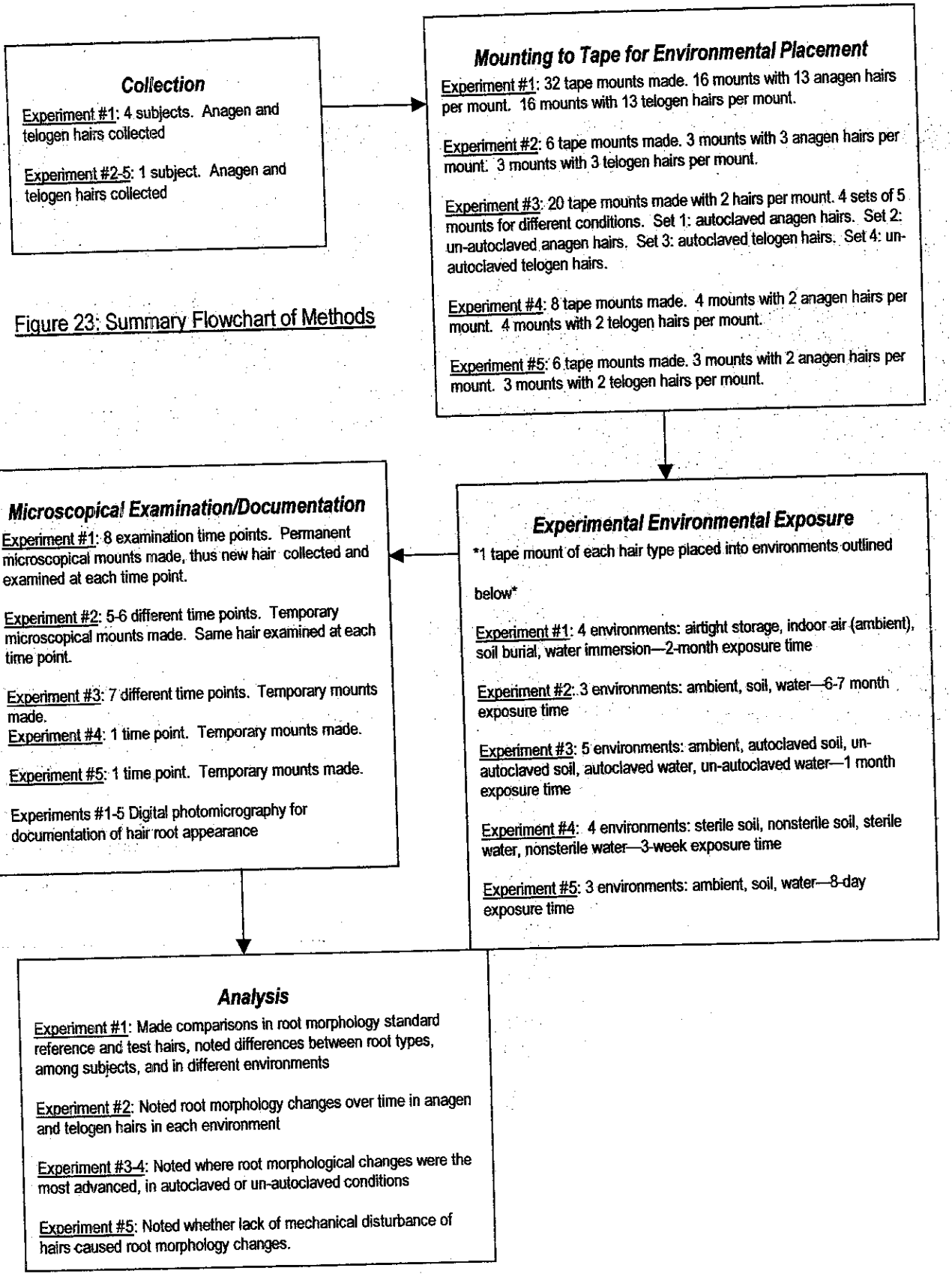

Figure 23: Summary Flowchart of Methods

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